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CONTRIBUTIONS TO EMBRYOLOGY, NO. 109

EARLY STAGES IN THE DEVELOPMENT OF PIG EMBRYOS, FROM THE PERIOD OF INITIAL CLEAVAGE TO THE TIME OF THE APPEARANCE OF LIMB-BUDS

By C. H. Heuser and G. L. Streeter

Department of Embryology, Carnegie Institution of Washington

With twelve plates and eight text-figures

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CONTENTS

	PAGE
Introduction	3
Material and Methods	3
Process of Cleavage	10
One-cell Egg	11
Two-cell Egg	12
Four-cell Egg	13
Eight-cell Egg	14
The Blastocyst	15
Formation of the Segmentation Cavity	15
Inner Cell-mass	16
Entoderm Cells	18
Germ-disk	20
Formation of Elongated Chorion	21
Pathological Chorions	21
Development of the External Form of the Embryo	22
Primitive Groove	22
Neural Groove	22
Formation of Amnion	23
Somites, Ear, Eye	24
Allantois	25
Closure of Anterior Neuropore	25
Branchial Arches	26
Summary	27
Bibliography	29

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EARLY STAGES IN THE DEVELOPMENT OF PIG EMBRYOS, FROM THE PERIOD OF INITIAL CLEAVAGE TO THE TIME OF THE APPEARANCE OF LIMB-BUDS

INTRODUCTION

When Professor Keibel (1897) brought out the first number of his series of "Normentafeln zur Entwicklungsgeschichte der Wirbelthiere" he chose the pig as the introductory animal, and since then one would not think of doing any embryological work upon this form without the Keibel volume close at hand. It is therefore with a feeling that a word of explanation is due that the authors of the present paper undertake a publication that partakes of the nature of an atlas on the development of the pig embryo. It is to be at once pointed out that the accompanying plates are arranged in the form of, and in a sense constitute, a photographic atlas of the principal steps in development of the egg and early embryo, but at the same time they are subservient to the text of the paper which gives but a running description of certain phases in development and is in no sense encyclopedic. We have tried to trace the general form of the embryo, without any considerable gap, from the one-cell stage up to the time of the appearance of limb-buds. It has been necessary to consider cleavage and histogenesis, but for the greater part our attention has been directed to the features of gross morphogenesis. The wealth of material to which we have had access has made it possible to present an account of stages earlier than those covered by Keibel, and in the overlapping stages greater detail has been secured. Thus it is our hope that this paper will prove useful as a supplement to Professor Keibel's classical work.

Modern photographic methods have made it possible to improve greatly the character of morphological records, and they are of particular value in these small embryos that have to be studied under the microscope. By having many photographs of closely grouped stages lying before one, where they can be readily compared, oftentimes features come out that would otherwise pass unnoticed. The great value of the camera has been shown by Hill (1910) and by Hartman (1919) for marsupials and now in our turn we have used it freely for a higher mammal. The entire credit for their excellence belongs to the younger of the two authors.

In carrying on this joint investigation there has been a partial division of labor, although for the greater part there has been close counsel and conclusions jointly arrived at. Two sections of this study have already appeared, one describing the implantation of the ovum by Heuser (1927) and the other on the development of the mesoblast and notochord by Streeter (1927). They were published separately as a matter of convenience, but should be regarded as belonging to the present paper.

MATERIAL AND METHODS

Many disadvantages might be mentioned as to the site of the Johns Hopkins Medical School, in which the Carnegie Embryological Laboratory is located. It has, however, one advantage that is of great importance to the embryologist,

namely, its close proximity to a pork-packing establishment, where pigs are slaughtered daily and where the fresh, warm uteri can be obtained in abundant quantity. Embryological material thus obtained from the pig has played an important part in the teaching and researches of the anatomical department from the time the department was started under the leadership of the late Professor Franklin P. Mall. Although it was always possible to secure living or recently dead embryos of all sizes above five millimeters from this source, yet difficulty was met with in finding the earliest stages. These were sought for, but over a period of many years were not found. It was not until the researches of Dr. G. W. Corner had taught us the macroscopic changes in the ovary and their relation to the œstrous cycle that it was realized that the early eggs and embryos must be present but were being overlooked. Following Dr. Corner's leadership, we learned how to wash out the tubes and uteri and readily obtain the stages in development which we had so long been seeking. In the course of one winter we obtained material of all sizes, from small blastocysts less than a millimeter in diameter, up to embryos in which the anterior and posterior neuropores had closed and the limb-buds could be definitely recognized. Since this period of development is so poorly represented in the human embryo, it has seemed that a careful study of pig material would be justified, biding the time when these early developmental processes can be corroborated in primate ova.

For the very earliest stages our source of supply was not adequate. The pigs for the greater part are shipped to this particular abattoir by rail, and consequently their history during the few days preceding slaughter is unknown. Though a sow may be in heat during that time, there is small chance of the eggs becoming fertilized, owing to the practice of castrating young males. Even in the few cases where small fertilized eggs were found, there were no data as to their exact age. At this juncture we were greatly aided by the generous cooperation of Dr. Sewall Wright (now at the University of Chicago) and Dr. Hugh C. McPhee, of the Bureau of Animal Industry, of the U. S. Department of Agriculture. At the Government experimental station at Beltsville, Maryland, they were able to have sows bred and subsequently slaughtered at desired periods. In this way we came into possession of eggs of which the copulation age was exactly known. Altogether, we have had the opportunity of studying 14 litters (74 eggs) of the first week, varying from slightly under two days old up to just over seven days old. In the first litters many of the eggs were lost while our technique for obtaining them was being perfected. For convenience in subsequent reference there is given, on page 5, a tabulation of this material of the first week of development.

In order to obtain tubal ova resort was had to the same technique used by Corner (1921), consisting of washing out the Fallopian tubes with a stream of isotonic salt solution. It is first necessary to straighten the tube by separating it from its mesosalpinx. The tube is then detached, along with the tip of the cornu. We have found it convenient to wash out the tube by using a large rubber bulb (60 c.c. capacity) to which is attached a glass pipette of wide diameter with the free end tapering rapidly to an opening of about 3 mm. The bulb then is filled with salt

solution and the fimbriated end of the tube is pulled upon the cone-shaped end of the pipette and held firmly in place around it. When pressure is exerted on the bulb the tube is immediately distended and the fluid carrying the eggs escapes from the cut uterine end into a small watch glass. Usually all of the ova come away with a single washing. It may, however, be necessary to repeat the washing if one wishes to be sure of recovering all of them. On inspecting the washings under a binocular microscope one soon learns to recognize the ova. In carrying out this procedure it is well to use as little fluid as possible, so that there will be a minimum volume of fluid to search through. The ova sink to the bottom of the dish and, if the bottom presents a concave surface, they will tend to collect toward the center of it. In such washings there is a considerable amount of débris which is a hindrance in the location of the specimens. When one learns what to expect, and also the characteristic refractive appearance of the zona pellucida, there is little difficulty in identifying the eggs. In this procedure the use of a black background is advantageous, and also one should have a beam of light that can be played on the fluid for direct illumination of the eggs.

Our No.	Beltsville No.	Stage	Number of eggs	Fertilization age
388	29.5	1-cell	4 tubal	2 days 3 hours
286	60	2-cell	3 tubal	2 " 3½ "
285	1	4-cell	3 tubal	1 " 20 "
389	29.2	3–4-cell	2 uterine	3 " 3 "
288	850	4±-cell	5 uterine	3 " 2 "
289	820	.....	6 uterine	3 " 2 "
281	82 Ga.	6–8-cell	6 uterine	3 " 18 "
390	95.9	12±-cell	1 uterine	3 " 3 "
391	15.1	60±-cell (morula)	3 uterine	1 " 23 "
284	66	Morula	11 uterine	4 " 3½ "
291	63	Segmentation cavity	7 uterine	4 " 18 "
287	82	Blastocyst inner cell-mass	8 uterine	6 " 1¾ "
282	84	Blastocyst inner cell-mass	4 uterine	7 " 3½ "
283	88	Larger blastocyst with endoderm cells	11 uterine	6 " 20 "

For washing out uteri the rubber bulb is replaced with a gravity bottle since, owing to the size of the uterine lumen, it is necessary to use much more fluid. The uterine cornu is first freed of its mesometrium and separated from the cervix. A large hæmostatic clamp is then applied to its cervical end. By inserting a large glass canula at the tubal end, warm saline solution is allowed to flow under gravity pressure, distending the whole canal moderately. Releasing the clamp, the contents of the cornu are allowed to flow into a glass finger bowl. Holding up the cornu, the fluid is apt to rush out rapidly, and one must use care to avoid losing some of it in this way. Usually, one finger bowl is large enough to receive the washings. It is well to have a second dish at hand to receive the excess in case of a large specimen. One washing of this character is almost invariably sufficient to remove the entire content of the uterine horn. In a few instances we used a fixative solution as a washing agent, for the purpose of fixing the eggs immediately in their natural environment. There is no difficulty in carrying out this procedure, but it was found unnecessary, since we could see no difference between

the eggs fixed in that manner and those that were temporarily in isotonic salt solution.

When we first began to study these eggs, we fixed them before recording them photographically. Experience taught us that we could secure better records with the camera if photographs were made while they were still in the saline solution. They should at least be studied in the fresh condition. When one is ready to transfer them to the fixative they should be handled by the use of a glass pipette drawn out to a long slender capillary tube. The egg is not drawn up into the chamber of the pipette, but is only sucked up into the capillary portion. It should be handled under the binocular with the egg kept always in view. As a fixative, Bouin's fluid and corrosive acetic were resorted to, both of which gave good results. Larger eggs, such as those found in the uterus, were always more or less wrinkled. This seems to be their normal form; they appear in this condition even when

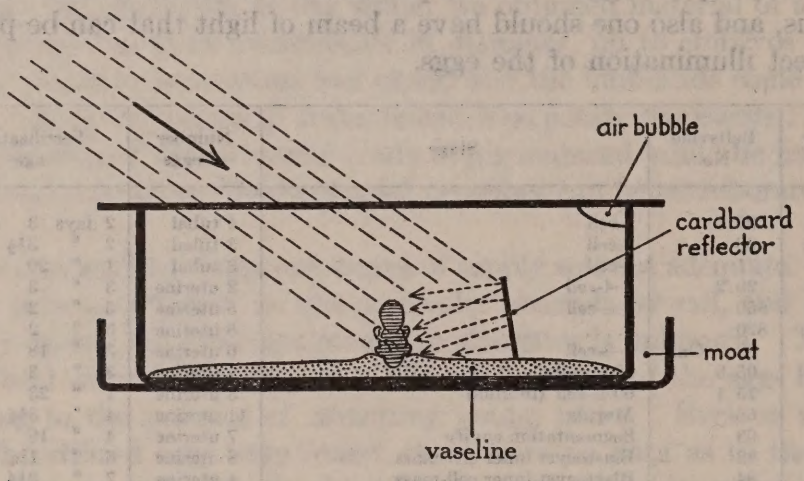


FIG. 1—Glass chamber in which embryos are photographed under fluid. It is usually best to illuminate the specimen obliquely from above with a beam of parallel rays, with some of them reflected to the shadow side.

fixed *in situ* by the injection of fixing fluid into the uterus. They can be distended with saline solution using a fine glass needle and brought to a spherical condition and then hardened by depositing fixative over them from a pipette, but it is to be remembered that this is probably not the normal form and there is some danger of displacing the endoderm by thus filling the bilaminar blastocysts with fluid. As the eggs get larger it becomes desirable to preserve them so that no folds occur in the region showing the embryonic disk. The adjustments should be made in salt solution by using a stream from the pipette or by manipulation with needles. As soon as the egg is satisfactorily placed, a stream of Bouin's can be gently flowed over the area, which immediately fixes it in position. In handling the elongated ova after they had grown into the thread-like condition, it was usually found that they were expelled from the uterus in an intricately snarled mass. As a rule, no attempt was made to unravel these specimens, the central portion alone of each chorion being cut out and preserved. To obtain the ova in this stage intact and

detached from other ova, it is desirable to open the straightened uterus under saline solution and cause the entire chorion to float free.

In studying this embryological material, special efforts were made to secure adequate photographic records, both of the early eggs and of the embryo itself. For the larger specimens a shallow Stender dish with a glass cover was utilized, as shown in text-figure 1. The dish is completely filled with distilled water or alcohol and the cover slid on in a way to let the last air bubble escape. In the bottom of the dish there is placed a glass disk covered with black vaseline which serves as a contrasting background and also makes it possible to accurately hold the embryo in position. This posing of the specimens is done by raising up or adding small bits of vaseline with needles to form appropriate supports for it. The dish should also contain a curved white cardboard reflector, which is placed just outside the field covered by the lens, for illuminating details in the shadows.

When the specimen is installed in this manner a conical beam of light is thrown obliquely through the cover of the dish so as to illuminate the surface of the embryo, bringing out in relief its delicate surface modeling. The principal object of the cover to this dish is to prevent any movement of the contained fluid. A special photographic equipment has been gradually assembled in this laboratory, which greatly facilitates the proper placing and illumination of these specimens. This equipment is illustrated in text-figure 2. It possesses certain desirable features. A binocular microscope and the camera are mounted on hinged supports so that when they are moved alternately into position their optical axes will be identical. Hence after the specimen has been adjusted under the microscope its image will fall centrally on the photographic plate. The board carrying the camera is hinged to another, supported at the top by a clip which rides over the arc and secured at the lower end by a bolt which serves as a pivot on which it can be tilted to the right and left for stereophotography. The arc through which the top of the board moves is calibrated with equidistant holes on either side of the upright position, and pegs are inserted at the desired angles for the two images. The specimen is always placed at the level of the pivotal bolt so that the two images will in each case fall centrally on the plate. The placing of the specimen at this correct level is facilitated by mounting the microscope with its focus fixed on the level of the pivot so that when the specimen is observed under the microscope and brought up to its focus, by means of a stand provided with a rack and pinion, it will be in the proper horizontal plane. The viewing microscope is then swung to the side and the camera moved into position above the specimen. The various objectives and the level of the plate holder are graduated in such a manner that any desired magnification can be secured by adjustment of the length of the bellows and level of the camera as a whole to a series of marks engraved on the side of the apparatus. The illuminating device consists of a 6-volt, 108-watt, gas filled Mazda lamp, provided with a Bausch and Lomb aspheric condenser. By focusing the condenser a beam of light of almost any desired intensity can be obtained. One portion of the specimen is brilliantly illuminated by the lamp and the opposite surface is adequately shown by the reflex light cast by the cardboard reflector in the fluid chamber. The lamp

rests on a shelf which is independent of the pedestal which holds the focusing stand, so that movements of objects on the shelf do not set up tremors in the dish containing the specimen. In order to obtain a firm pedestal, a wooden case was made in the form shown in text-figure 2, and filled with sand.

For securing records of small eggs, the focusing stand is replaced by the ordinary compound microscope, so that the specimen may be illuminated with transmitted light. The eggs are placed on a 1 × 3 microscopic slide (in some cases a hollow-ground slide was used) rimmed with vaseline and covered with a cover glass. It was found that a hair could be left projecting through the vaseline ring, and by means of it the eggs could be adjusted as to position without disturbing the cover.

A word might be said as to the method of getting the eggs, one at a time or in groups, sealed in the vaseline ring. If the attempt is made to place a cover over the specimens in the filled ring the result will probably be a great disturbance in their arrangement and doubtless also the escape of some of the eggs in the excess fluid which runs over the sides of the chamber. An easy and safe method is to submerge the slide carrying the vaseline ring in a dish containing the same fluid in which the eggs are to be mounted. The specimens are then arranged and the cover slowly lowered into position and pressed gently on the vaseline. When the slide is removed from the dish and the cover carefully dried with bits of filter paper it is usually found that the specimens have not changed their positions from the time when they were first placed in the ring. With such a mount the eggs may be illuminated by either transmitted or direct light. In using transmitted light, better results were obtained on fresh eggs than on fixed material. Fixation makes them so opaque that one sees little more than the contour of the constituent cells, even after they have been stained and cleared.

Various methods of embedding and sectioning small eggs may be used, such as coagulating them in an albumen mass or placing them in a bed of tissue, or even cutting them *in situ* along with the tube or uterus. Such procedures, however, do not make it possible to orient the object. Orientation is extremely important. It was therefore found necessary to resort to a technique in which the orientation of the eggs under the microscope could be controlled. By utilizing a long-drawn out pipette and passing the eggs from one dish to another under the binocular microscope, there was no difficulty met with up to the time of placing the egg in mixtures of xylol and paraffin. But heated instruments are required from this stage on until the specimen is embedded. It was found that the eggs could be transferred from one paraffin dish to another by utilizing a pipette wound with resistance wire and heated with the electric current of just the correct voltage to keep the contained paraffin at the melting point. During all the manipulation of the paraffin one should have a convenient apparatus for holding the dishes at the proper temperature. We used a brass plate electrically heated to a constant temperature, and in addition overhead heat was obtained by radiation from electric bulbs. Over a portion of the hot plate a binocular microscope was installed so that the dish and specimen could be observed; all the changes from one fluid to

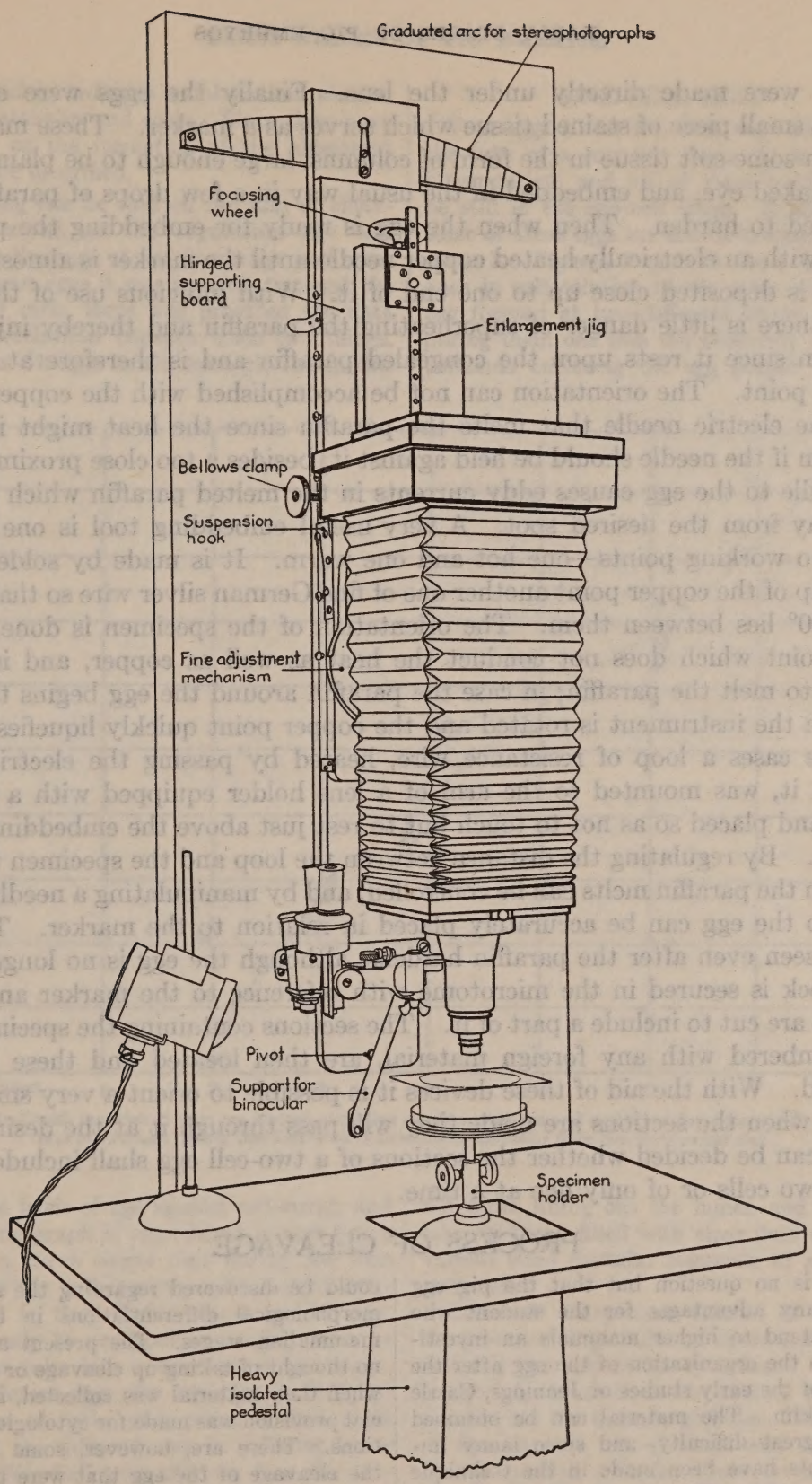


FIG. 2—Drawing of apparatus used for photographing small embryos. About 1/12 actual size.

another were made directly under the lens. Finally the eggs were embedded beside a small piece of stained tissue which serves as a marker. These markers are cut from some soft tissue in the form of columns, large enough to be plainly visible to the naked eye, and embedded in the usual way in a few drops of paraffin which is allowed to harden. Then when the egg is ready for embedding the paraffin is melted with an electrically heated copper needle until the marker is almost free and the egg is deposited close up to one end of it. With judicious use of the electric needle there is little danger of superheating the paraffin and thereby injuring the specimen since it rests upon the congealed paraffin and is therefore at about its melting point. The orientation can not be accomplished with the copper point of the same electric needle that melts the paraffin since the heat might injure the specimen if the needle should be held against it; besides a too close proximity of the hot needle to the egg causes eddy currents in the melted paraffin which carry the egg away from the desired spot. A very useful embedding tool is one provided with two working points—one hot and one warm. It is made by soldering near to the tip of the copper point another one of fine German silver wire so that a gap of about 90° lies between them. The orientation of the specimen is done with the silver point which does not conduct the heat as well as copper, and is not hot enough to melt the paraffin; in case the paraffin around the egg begins to congeal too soon the instrument is rotated and the copper point quickly liquefies it again. In some cases a loop of resistance wire, heated by passing the electric current through it, was mounted to the arm of a lens holder equipped with a rack and pinion and placed so as not to touch but to rest just above the embedding mass of paraffin. By regulating the distance between the loop and the specimen the depth to which the paraffin melts can be controlled, and by manipulating a needle through the loop the egg can be accurately placed in relation to the marker. The latter can be seen even after the paraffin hardens, although the egg is no longer visible. The block is secured in the microtome with reference to the marker and enough sections are cut to include a part of it. The sections containing the specimen itself, unencumbered with any foreign material, are then located and these alone are mounted. With the aid of these devices it is possible to orient a very small object so that when the sections are made they will pass through it at the desired plane; thus it can be decided whether the sections of a two-cell egg shall include portions of the two cells or of only one at a time.

PROCESS OF CLEAVAGE

There is no question but that the pig egg offers many advantages for the student who would extend to higher mammals an investigation on the organization of the egg after the manner of the early studies of Jennings, Castle and Conklin. The material can be obtained without great difficulty, and since many improvements have been made in the technique of fixing and handling the material, it is very probable that in this old field many new things could be discovered regarding the sequence of morphological differentiations in the earliest mammalian stages. The present authors had no thought of taking up cleavage or cell-lineage when their material was collected, and no special provision was made for cytological observations. There are, however, some features in the cleavage of the egg that were plainly evident in our study of the gross changes, such as can be seen in the entire egg under low magni-

fications and in ordinary serial sections stained with hæmatoxylin and eosin. Since they have a bearing on the formation of the blastocyst they will be described.

Something can be said on the rate of cleavage. Inasmuch as the copulation age was known for the eggs of the first week and since in these same eggs it was possible to count the number of cells, there was provided the data for the correlations between these two things. Ten litters, 41 eggs in all, were plotted in this way on the basis of age against cell-count, and the resulting graph is reproduced in text-figure 3. Such a curve shows that during the first seven days the cells undergo about eight divisions, that is, they divide about once a day. This can only be accepted as roughly true, as it does not take into consideration the variation in different eggs, the variation in different generations of cells, and, what is still more important, the different rate in different kinds of cells. We shall presently see that the trophoblast divides more rapidly than the rest of the egg.

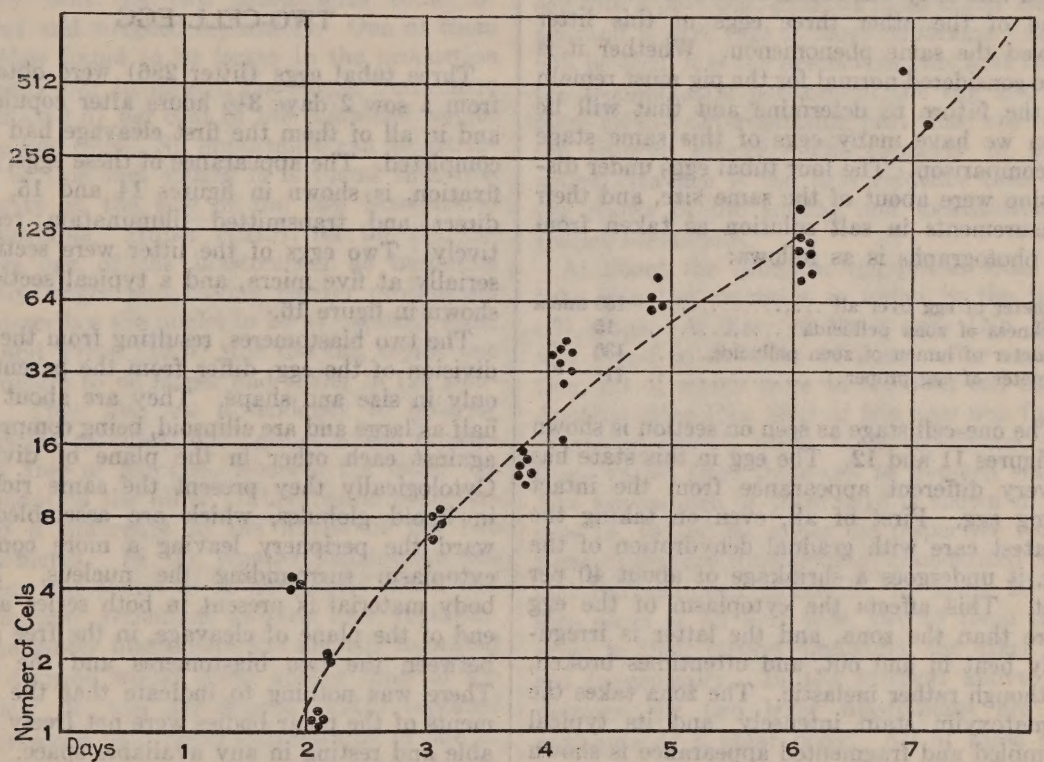


FIG. 3—Graph in which 41 eggs are plotted according to copulation age and number of component cells. The rate of cleavage during this first week is thus not much faster than one division per day.

ONE-CELL EGG

Four eggs (litter No. 388) were washed from the tube of a sow two days (51 hours) after insemination, and all of them were found to be still in the one-cell stage. Photographs of some of these eggs are shown in figures 9 to 13, plate 1. Figures 9 and 13 show the appearance in salt solution of the same intact living egg focused at two different levels. By comparing them one sees the relatively thick zona pellucida enclosing the egg proper, the latter not quite filling out the lumen and the small interval being filled with clear fluid containing various sized granular remnants of polar body material.

The most conspicuous feature of the egg is the presence of globules of lipoid material distributed throughout its cytoplasm. In figure 9 which is focused on the surface of the egg one can see the discrete globules. The borders of the egg are out of focus and due to the closely packed globules there is produced a false appearance of a light space surrounding it. The true periphery of the egg is just out-

side this refractive zone. The fat droplets vary in size, the larger ones tend to lie at the periphery of the egg and are uniformly distributed over its surface. No tendency could be recognized toward a polar accumulation like that described by R. van der Stricht for the cat.

The egg shown in figure 10 is interesting in that it is characterized by a large body that appears to consist of extruded yolk and cytoplasm, reminding one of the *Dasyurus* eggs pictured by Hill, 1910. When the egg was sectioned this body was found devoid of chromatin. None of the other three eggs of this litter showed the same phenomenon. Whether it is to be considered normal for the pig must remain for the future to determine and that will be when we have many eggs of this same stage for comparison. The four tubal eggs under discussion were about of the same size, and their measurements in salt solution as taken from the photographs is as follows:

Diameter of egg over all	160	micra
Thickness of zona pellucida	15	"
Diameter of lumen of zona pellucida	130	"
Diameter of egg proper	111	"

The one-cell stage as seen on section is shown in figures 11 and 12. The egg in this state has a very different appearance from the intact living egg. First of all, even on taking the greatest care with gradual dehydration of the egg, it undergoes a shrinkage of about 40 per cent. This affects the cytoplasm of the egg more than the zona, and the latter is irregularly bent in and out, and oftentimes broken, as though rather inelastic. The zona takes the hæmatoxylin stain intensely, and its typical crumpled and fragmented appearance is shown in figures 11, 16 and 20. The cytoplasm of the egg, in its shrunken and coagulated condition, is rather constant in arrangement. It is rather denser in the perinuclear region and around the periphery of the ovum where it constitutes a bordering membrane sometimes designated as the vitelline membrane. Apparently this latter is not a discrete cuticle that could be stripped off from the adjoining cytoplasm, but merely the cytoplasm itself thickened in the process of shrinkage and coagulation. Between the narrow border zone and the condensed central field one sees everywhere the lacunæ from which the lipoid droplets have been dissolved. The larger droplets are more toward the surface and smaller ones toward the center. The stained preparation presents the appearance of a granular network of coagulated cytoplasm, which in the living must have been streaming protoplasm between the lipoid droplets, perhaps, of varying density. Finally, as for the nucleus in these sections of the one-cell stage, one finds the nuclear membrane absent or poorly defined and the chromatin material exhibiting various phases of nuclear division. In figure 11 the chromosomes are arranged in a cleavage spindle and the division of the egg into two cells is imminent.

TWO-CELL EGG

Three tubal eggs (litter 286) were obtained from a sow 2 days 3½ hours after copulation and in all of them the first cleavage had been completed. The appearance of these eggs, after fixation, is shown in figures 14 and 15, with direct and transmitted illumination respectively. Two eggs of the litter were sectioned serially at five micra, and a typical section is shown in figure 16.

The two blastomeres, resulting from the first division of the egg, differ from the parent cell only in size and shape. They are about one-half as large and are ellipsoid, being compressed against each other in the plane of division. Cytologically they present the same richness in lipoid globules, which are assembled toward the periphery leaving a more compact cytoplasm surrounding the nucleus. Polar body material is present in both series at the end of the plane of cleavage, in the free space between the two blastomeres and the zona. There was nothing to indicate that the fragments of the polar bodies were not freely movable and resting in any available space.

Numerous sperm heads are found in the zona pellucida. When the zona is stained too intensely they can not be seen. In one of the series (286–1) the zona was tinged with hæmatoxylin only slightly, whereas the sperm heads were stained deep blue, and they could thus be readily recognized. They invariably lie with their flat surface in the plane of the zona and for the most part near the surface. They thus are shown in transverse or sagittal section and appear as slender rods. Where the zona is cut tangentially a flat view of the sperm heads can be had. Under such circumstances the tongue-shaped nucleus can be identified, the caudal rim of which is deeply stained and in some cases notched producing two rounded tips.

Eighteen sperm heads were counted in this one series, and as the zona was imperfect in some places there were doubtless a larger number adhering to the egg before sectioning.

One would like to find at this stage a difference in structure of the two cells, in support of van Beneden's theory, advanced for the rabbit, that they perform clearly separate rôles in the subsequent formation of the blastocyst. The only suggestive evidence in our material was an inequality in their size. A wax-plate model was made at 400 diameters enlargement in such a way that the two blastomeres could be studied and weighed separately. One of them was thus found to be larger in the proportion of about four to three. The larger blastomere constituted 57 per cent of the combined weight of the two cells. No importance can be attributed to this fact unless it is found that this disparity in the size of the two blastomeres is invariable. Huber (1915), for the rat, describes one of the blastomeres as becoming slightly larger and having clearer protoplasm and describes the nuclei to be at opposite ends. Hill and Tribe (1924), for the cat, report one blastomere to be larger and richer in fat globules. They also see a tendency to polarity of the fat globules; one pole of which is relatively free of globules, whereas the latter are abundant in the other. These same observers found a difference in the nuclei of the two blastomeres. In the smaller blastomere the nucleus was found to be irregularly lobed and the nuclear reticulum swollen and vesicular. In the larger blastomere the nucleus indicated that division would occur sooner than in the smaller. R. van der Stricht (1911) reported the presence of a *corps énigmatique* in one of the blastomeres, a spherical body with deeply staining membrane enclosing a lighter area with several granules. Hill and Tribe also saw a similar structure, but they found that it was present in both blastomeres. We have looked for these differences in the pig, but in the small number of eggs we have for study we can not find them. The nuclei appear to be essentially alike in the two cells and one can not say which was destined to divide sooner. Nor is there any evident difference in the cytoplasm. It should be added that in the groove between the two cells there is a small amount of finely granular material, as described by Hill and Tribe. They regard it as material eliminated by the blastomeres.

FOUR-CELL EGG

With the completion of the separation of the first two blastomeres there has doubtless occurred some sorting of the constituent substances of the egg and the two blastomeres are probably not identical. Even though no histological evidence of this could be seen, it must be assumed from what is known of eggs in lower forms. Furthermore, one of the blastomeres is more precocious in the onset of the second cleavage, giving rise to a three-cell egg. From this time on one can divide the egg into a part that exhibits more active cleavage and a part that is more deliberate. As it will be seen, it is the precocious part that forms the trophoblastic portions of the egg and the more slowly differentiating part that will form the embryo proper. The property of more active cleavage is the first evidence that this egg shows of the sorting and localization of special substances.

At about the time the egg passes from the tube into the uterus it is found in the four-cell stage. A litter of three eggs (No. 285) was found in the tube 44 hours after copulation, all of which were in the four-cell stage. Another litter (No. 288) of five eggs was found in the uterus 74 hours after copulation, and these varied from three-cell to five-cell stages. The appearance of these eggs is shown in figures 18, 19 and 20, under the respective conditions of surface illumination, transmitted light and the sectioned material. In figure 20 one sees parts of all four cells, two of them traversed in their largest diameters, the other two cut tangentially. In the interval above and between the two large blastomeres are the remnants of a polar body. All of these eggs were cut in serial sections and a careful microscopical study of them failed to reveal any obvious structural difference in the four blastomeres, other than nuclear changes associated with their varying states of division. They are primitive in type and repeat the structural design of the single-cell stage.

However, when one reconstructs models of eggs in the three-, four- and five-cell stages, it is found that the blastomeres in this process begin to differ in the matter of sizes. Even in the two-cell stage the blastomeres differed in size. In egg No. 288–B one of the first blastomeres has divided, the cleavage plane intersecting the preceding first cleavage plane at right angles. A wax-plate model of this egg,

text-figure 6*b*, shows that the larger of the two blastomeres had divided, producing an egg of three cells, whose respective volumes were 45, 29 and 26 per cent of the whole. In another egg of the same litter (No. 288–A) the other primary blastomere had divided giving four cells, having a volume percentage of 30, 20, 30 and 20 per cent. In each cleavage the "halves" tend to be unequal and the amount of inequality presumably varies from egg to egg. The question of volume proportion of the early blastomeres can be better taken up after we have reached the eight-cell stage.

The appearance of the egg in the intermediate period as it is passing from the four-cell to the eight-cell stage is shown in figures 17 and 21. These were photographed in the living state in normal salt solution.

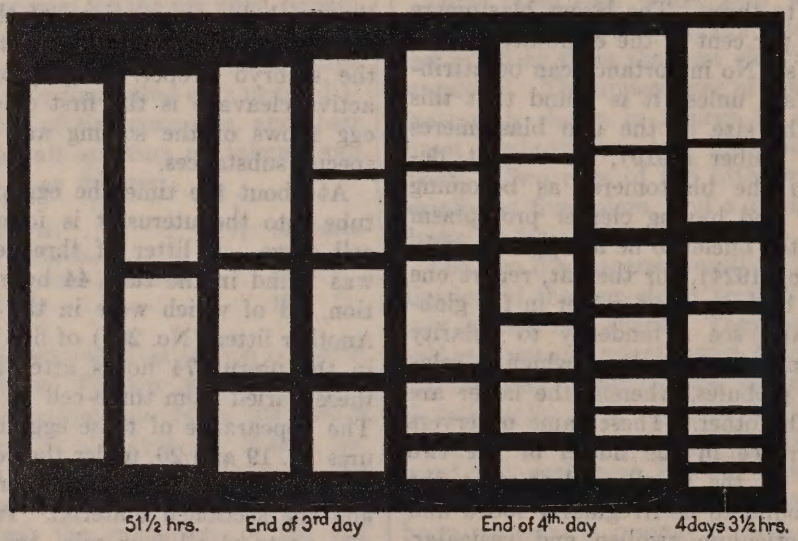


FIG. 4—Diagram showing the segmentation of the mammalian egg (pig) based on the proportionate volume of the component cells up to the eight-cell stage. The disparity in cell-volume and in the rate of subdivision is interpreted as an expression of the localization and sorting-out of particular substances and qualities before these are histologically recognizable. It is in that way that the trophoblastic part of the egg becomes separated from that which will form the embryo proper.

EIGHT-CELL EGG

A litter of six eggs (No. 281) was obtained from the uterus of a sow at the end of the fourth day (3 days 18 hours) after copulation. Of these one was abnormal, two were injured in the technique of sectioning, and the remaining three were successfully carried through in serial sections as normal specimens having respectively five, six and seven cells. A photograph of one of these eggs is shown in figure 22, and a section of egg, No. 281–3, having 6 cells, is shown in figure 23. In structure the cells still retain their primitive characteristics. One can see, however, that they show some discrepancy in size. Reconstructions were made of all these eggs, and their proportional volume constitution was as follows:

No. 281–1, five cells, 30—26—20—14—10 per cent.
No. 281–3, six cells, 25—19—19—14—12—11 per cent.
No. 281–4, seven cells, 29—17—15—13—13—7—6 per cent.

From these figures and a study of the models one can see that the discrepancy in size is the result of more active division of some cells than others. In the seven-cell stage there is represented the fourth, third and second generations. The more actively dividing cells can be traced as those that are to form trophoblast in the succeeding stages.

By the beginning of the fifth day one finds that the egg has divided into eight or more cells. The eight-cell egg (No. 284–9) presents a striking diversity in cell size, the one modeled being made up of the following cell components: 31, 18, 16, 11, 11, 5, 4 and 4 per cent. It should be explained that these percentages are to be taken as only approximate because of a large possible error in determining them and also because of the unknown extent of variation from egg to egg. The egg used as representative of the eight-cell stage was cut in

a five-micra series of 11 sections. These were traced at a magnification of 400 diameters on two-millimeter wax-plates. When these are cut out the edges are necessarily step-like and in the case of the smaller cells having but three plate-layers, there is introduced considerable inaccuracy. When it is found that a given cell constitutes four per cent of the egg volume, the true volume might be as much as five per cent or perhaps three and one-half per cent. The purpose of reporting the volume data is to show the trend in the mechanism of division rather than emphasizing exact percentages.

If one schematically plots the cell volumes up to the eight-cell stage the result will be as shown in text-figure 4. The outstanding feature in the cleavage of the pig egg is the disparity in volume that soon shows itself in the component cells, and correlated with this is a definite difference in rate of subdivision of different parts of the egg, the part that is to be trophoblast undergoing more precocious subdivision than the part that will form the embryo. This difference in cleavage rate is the only obvious sign of localization of special substances and qualities in egg cytoplasm. The sorting-out of the special substances may very well be the explanation of the disparity in size that is so noticeable in the "halves" with each cell division. See text-figure 6.

THE BLASTOCYST

FORMATION OF THE SEGMENTATION CAVITY

Up to the 16-cell stage the only thing that clearly distinguishes some cells from others is their size which in turn means their activity in dividing. At about that time, however, the actively dividing cells undergo differentiation in form and one can recognize them morphologically as trophoblast cells. This period is represented by a litter of 11 uterine eggs obtained 4 days 3½ hours after copulation (litter No. 284). Coincident with the morphological specialization of the trophoblast cells there occurs the beginning of the segmentation cavity and its accumulation of fluid, the interesting first stages of which are found in this litter. These are the requirements necessary for the organization of the blastocyst, and it is not until this time that there appears to be any purpose in the mammalian egg of considering the matter of its polarization. Even in these early blastocysts the recognition of an anteroposterior axis of the egg is doubtful.

In the litter of eggs referred to one could not determine in their intact condition the beginning of the segmentation cavity or, with certainty, the early trophoblast cells. When these eggs were sectioned, however, it was found that the more advanced of the trophoblast cells had acquired identifying characteristics. This early form of the trophoblast cell is illustrated in figures 24 and 25. It will be seen that the cells marked "Tr." have flattened out against the zona and tend to form a membrane within which the first accumulation of segmentation-cavity fluid occurs. It seems safe to assume that there is a causative relation between the histogenesis of the trophoblast cell and the coincident fluid accumulation, and that the latter is evidence of functional activity of the trophoblast. That the flattening of the trophoblast cells is not simply the mechanical result of the collection of fluid is proven by the fact that in some eggs flattened cells were found unaccompanied by fluid pools.

Of this litter of 11 eggs, one was defective, being unsegmented and necrotic; another was ruined in handling. The remaining nine were suitable for study; four showed no evidence of the segmentation cavity, though possessing a few unmistakable trophoblast cells; one had a very early beginning cavity; three had small cavities, such as are shown in figures 24 and 25, and one had a large cavity, two sections of which are shown in figures 26 and 27. The latter egg (No. 284–8) is particularly interesting because of the clear demarcation between the characteristic trophoblast cells enclosing the segmentation cavity and the residual three large primitive cells from which the inner cell-mass will be derived. This demarcation can be well seen in figure 27 where two large relatively retarded cells are shown in the upper part of the photograph. The demarcation in this egg is made more evident by the fortunate plane in which the egg was sectioned. Knowing of no way of orienting eggs at this stage it was only by chance that the sections display so favorably the principal plane of separation between the trophoblast and the three large retarded cells. Serial tracings through this same egg are shown in text-figure 5 in which the trophoblast is shown in stipple and the large retarded cells are striated.

From the eggs in the litter under consideration there can remain no doubt as to the manner of formation of the segmentation cavity. There is a sufficient number of clearly normal specimens to abundantly confirm the essential picture of the process, and since the eggs slightly vary in the degree of transformation one is provided with a close series of stages. The picture is that of an accumulation of fluid in a pool in the intercellular spaces of the trophoblastic portion of the egg, and this accumulation does not begin until the trophoblast cells acquire certain histological characteristics. No evidence was seen of any breaking down or liquefaction of the central cells, and there seems to be no reason to resort to this as the source of the cavity or its contained fluid.

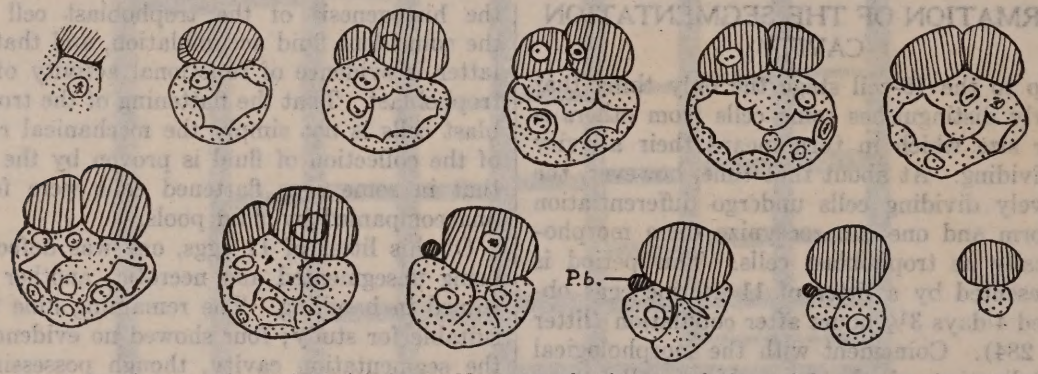
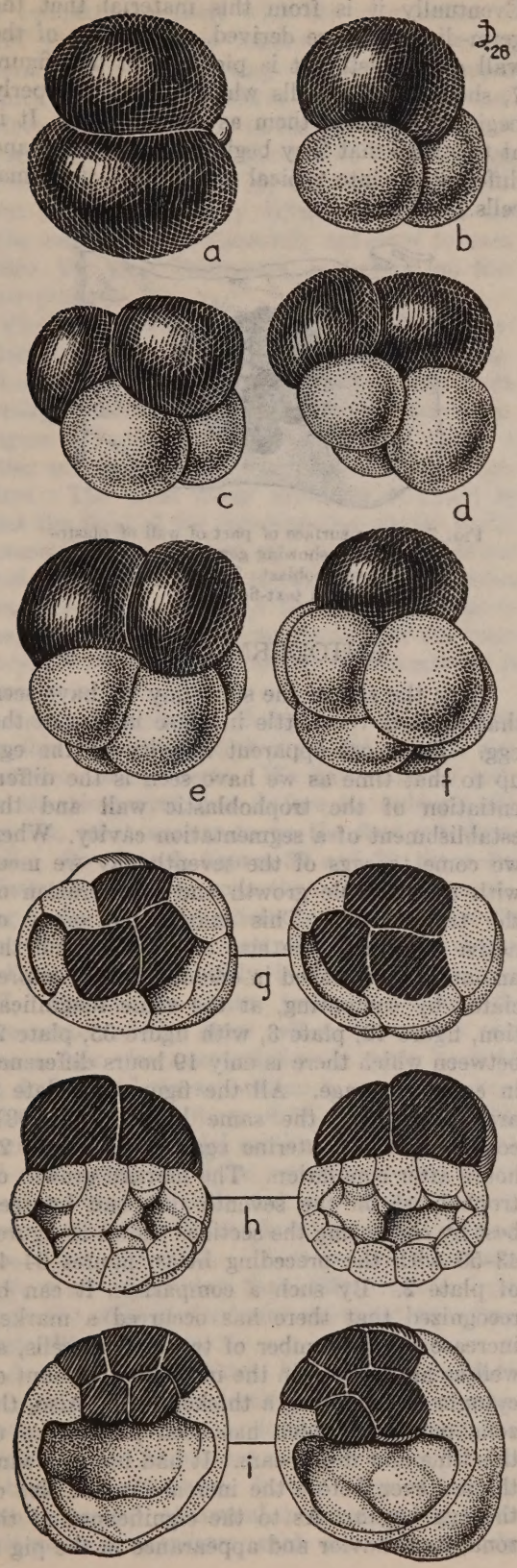


FIG. 5—Tracings of serial sections of a pig egg, No. 284–8, showing an early form of the segmentation cavity which at this time consists of a pool of fluid collecting within the trophoblastic portion of the egg (stippled). The entire egg consists of about 20 cells, and was obtained from the uterus 4 days 3½ hours after copulation. Sections 5 micra thick, magnification 300 diameters. The polar body appears in three sections (P.b.).

INNER CELL-MASS

By the time there are 30 or more cells the segmentation cavity can be distinctly recognized in the intact egg and one can begin to speak of it as a blastocyst. A litter of seven such eggs is shown in figure 28, plate 2. These eggs (litter No. 291) were obtained from the uterus of a sow 4 days 18 hours after copulation. Three of these eggs were less advanced, although on section it was found that they have a beginning segmentation cavity. The remaining four have well-developed cavities and one can recognize the outline of an inner cell-mass.

The surface anatomy of one of these early blastomeres is shown in figure 30. It shows the form and character of the trophoblast cells, and the oval opacity above reveals the contour of the inner cell-mass. To determine the character of this latter structure it is necessary to section the egg. Sections of three different eggs of this litter are shown in figures 29, 31 and 32. In the process of embedding it was possible to orient these eggs so that the sections pass transversely to the inner cell-mass, and those chosen for the illustrations were taken from the center and show the mass in its greatest thickness. In egg No. 291–7 (fig. 29), there are from eight to ten larger cells in the ventral part of the mass, and in addition there are two or three overlying them about to become trophoblast cells. These two types blend in the region of the inner cell-mass and one can not always separate them. In other words trophoblast cells seem to be still differentiating off from the group of large laggard cells which we have followed from the early stages of cleavage. When the last trophoblastic material has been budded off one can speak of the residuum as the true inner cell-mass from which in turn the entoderm and germ-disk cells will be derived. The section shown in figure 31 is from egg No. 291–5, and here we have six to eight large primitive type cells forming the ventral part of the inner cell-mass. Two are seen in the photograph, the protoplasm of one of which is retracted giving the false appearance of an intercellular space. Overlying these can be seen two cells which are flattening out to become trophoblast cells. The section shown in figure 32 is from egg No. 291–6, and here, in the inner cell-mass region, one can say that the separation of the trophoblastic layer is already indicated and the remaining cells are those that will form the germ-disk and its underlying entoderm. There is one free cell lying in the cavity which appears to be an early entoderm cell. It appears to have come from the inner cell-mass, for in



the next section it presents a thinned-out process which nearly connects it. The entoderm must arise in some such way, and it may be this is a correct picture of the first free entoderm mother cell.

The remaining photographs on plate 2 (figs. 33–40) are all taken from a litter of eight uterine eggs, 6 days 1¾ hours after copulation (litter No. 287). Seven of them are shown as a group in figure 33, and at the same magnification as those in figure 28. These two groups roughly represent the end of the fifth and sixth days, and by comparing them one can determine what the sixth day does for the eggs. It is chiefly an increase in size. The segmentation cavity is a little larger. There is not much change in the inner cell-mass, but there are more trophoblast cells. In the whole egg there are in most cases between 50 and 60 cells, whereas at the end of the fifth day there were between 30 and 40, and this increase is to be credited to the trophoblast.

Typical sections through the inner cell-mass of the six-day egg are shown in figures 34, 36 and 40. The number of cells in the mass is still small, at the same time there is evidence of cell division. This is explained by their continued contribution to the trophoblastic part of the egg. The inner cell-mass region remains the most primitive part of the egg, and in most all forms appears to continually differentiate off cells that join the trophoblast group. It constitutes a germinal mass of various potentialities. In figure 119, plate 12, are shown groups of eggs taken from various mammals, and the trophoblast cells are indicated in color. The general principles of a less differentiated inner cell-mass marked off from a more precocious trophoblast prevails in all of them. In many cells additional cells from the inner cell-mass are added to the trophoblast. When the trophoblast separation is complete, what is left of the inner cell-mass becomes the germ-disk and its underlying entoderm.

It will be noted that in this interpretation the inner cell-mass is not exactly the same

FIG. 6—Wax-plate models of the pig egg showing the formation of the segmentation cavity and the differentiations of trophoblast (stippled). The dark cells represent the more primitive part of the egg, and what is finally left of this material becomes the inner cell-mass and germ-disk. The distribution of color in eggs *a*–*c* is only introduced tentatively, for there is no reliable way for distinguishing trophoblast cells at that time. The eggs from which these models were reconstructed (×400) are as follows: *a*, 286–3; *b*, 288–B; *c*, 288–A; *d*, 281–1; *e*, 281–3; *f*, 281–4; *g*, 284–6; *h*, 284–8, and *i*, 291–5.

thing as the embryo, but something more than this. For a time it contains cells that are to become trophoblast, and until these are shed it is a composite or primitive germinal mass with the characteristics of the two-cell stage rather than of true ectoderm. In some mammals the separation of trophoblast from the rest of the egg is earlier and more complete than in others. In some marsupials it appears to be complete at the eight-cell stage (Hill, 1910, and Hartman, 1919). In rabbits it is early as judged by preparations shown me by Mr. P. W. Gregory of the Bussey Institution, whose paper I have had the privilege of seeing in manuscript form. In some rodents (rat, Huber, 1915) the trophoblast in the early blastocyst is like that of other mammals, but it soon undergoes a localized hypertrophy and builds the specialized implantation stalk. The pig and the sheep (Assheton, 1899) present the simplest arrangement of all the mammals, although the transformation into trophoblast is not so abrupt as in the lower mammals, and we thus see trophoblast cells still separating off from the primitive cell-mass material after the segmentation cavity is well established. The cat (Hill and Tribe, 1924) appears to be intermediate between the rabbit and pig. The bat (van Beneden, 1899) and *Tarsius* (Hubrecht, 1902) resemble the cat, although the trophoblast soon shows an active cell-proliferation indicating a future structure of more highly specialized character.

If one makes a series of wax-plate models of the pig egg, the steps in the precocious separating-out by cleavage and differentiation of the trophoblastic part of the egg can be plainly seen. Such a series is shown in text-figure 6. The trophoblastic part is shown in stipple. By the time the segmentation cavity makes its appearance the trophoblast cells are morphologically differentiated and unmistakable. Previous to that time they can be recognized, although with less certainty, by their active cleavage. Apparently one of the first two blastomeres becomes trophoblast, but one can not be sure of this, and the reconstructions are colored on that basis tentatively. It is not until we come to the five-cell stage that the size basis can be regarded as reliable, and even after the five-cell stage additional trophoblast appears to be derived from the primitive cells. These few slowly dividing larger cells, colored dark in text-figure 6, retain their primitive structure and form longer than the trophoblast. Eventually it is from this material that the germ-disk cells are derived. A portion of the wall of a blastocyst is pictured in text-figure 7, showing these cells when we may properly begin to speak of them as a germ-disk. It is at this time that they begin active division and differentiate into typical cylindrical ectodermal cells.

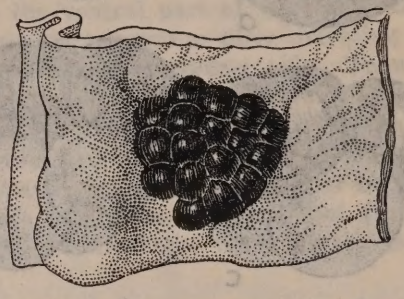


FIG. 7—Inner surface of part of wall of blastocyst (283–5) showing germ-disk cells underlying the trophoblast. This is a continuation of the series in text-figure 6.

ENTODERM CELLS

Up to the end of the sixth day we have seen that there is very little increase in size of the egg. The most apparent activity of the egg up to that time as we have seen is the differentiation of the trophoblastic wall and the establishment of a segmentation cavity. When we come to eggs of the seventh day we meet with a noticeable growth and proliferation of the trophoblast. This marks the onset of active growth in the size of the egg, and the amount accomplished in one day can be appreciated by comparing, at the same magnification, figure 42, plate 3, with figure 33, plate 2, between which there is only 19 hours difference in copulation age. All the figures in plate 3 are taken from the same litter (No. 283), consisting of 11 uterine eggs, 6 days and 20 hours after copulation. The marked growth of trophoblast on the seventh day can be seen best by comparing the sections shown in figures 43–50 with the preceding litter, figures 34–40 of plate 2. By such a comparison it can be recognized that there has occurred a marked increase in the number of trophoblast cells, as well as an increase in the individual amount of cytoplasm producing a thicker wall. Also, the zona pellucida which has been present up to this time now disappears. It had been growing thinner even before the increase in the size of the egg began. As to the significance of the zona, its behavior and appearance in the pig is

in complete harmony with the supposition of Hill (1910) that it constitutes a temporary membranous supporting structure against which the early primitive cells flatten themselves (steretropism ?) in the process of blastocyst formation, during the period while the egg is loosely articulated. By the time the blastocyst wall is sufficiently differentiated, and the cells completely and securely adherent to each other, the zona disappears, as having no further purpose.

Correlative with the expansion of the trophoblast wall is to be noted its marked folding. This is not a shrinkage effect, for eggs when freshly washed out show the same condition. Figure 41, plate 3, is a photograph of such a litter as seen under a magnification of 38 diameters. The most likely explanation would be that the fluid of the segmentation cavity is not accumulated rapidly enough to keep the blastocyst distended. The trophoblastic expansion outstrips the endosmosis (or whatever process may effect the fluid accumulation) of the blastocyst fluid, and consequently the membrane is thrown in folds.

Another important change in the egg occurs on this eventful seventh day—the definite appearance of entoderm. We have already seen in the blastocyst, just before its enlargement sets in, what appear to be the first entoderm cells. On plate 2, figures 32 and 39, there are illustrated solitary cells that are just detaching or are completely detached. Also figure 35 shows a cell on the ventral edge of the inner cell-mass, that possibly is about to separate off. The section passes transversely through the center of the inner cell-mass so that one has a fair view, nevertheless it must be held in doubt until the separation is more complete. Thus, of the two litters of 15 eggs, of the fifth and sixth days, serial sections reveal only two of them that could be seriously regarded as having entoderm cells and in both eggs it was only a solitary cell that had separated off from the inner cell-mass. If these are entoderm cells there is nothing that differentiates them from the cells of the inner cell-mass excepting their detachment.

As we come to the next litter, shown on plate 3, we meet with unquestionable entoderm composed of cells that are forming a lining membrane for the blastocyst and converting it into a bilaminar structure. In this litter of 11 eggs two appeared abnormal possessing no inner cell-mass or entoderm, each consisting simply of a folded trophoblastic vesicle, the structure of which appeared just like that of normal blastocysts. Another vesicle, the smallest one in the litter, has an inner cell-mass, but no entoderm cells have been given off yet (see fig. 49). The remaining eight blastocysts all have unmistakable entoderm cells, typical views of which are shown in the photographs on plate 3. In the more advanced specimens the entoderm cells are characterized by long connecting processes giving the appearance of an entodermal membrane. In less advanced specimens one finds many spherical entoderm cells devoid of processes and showing evidences of active division.

The evidence of the origin of the entoderm from the inner cell-mass rests first of all on the observations of Hill and Hartman who showed in marsupials the first entoderm cells arising in the formative area and their subsequent spreading over the whole inner surface of the blastocyst. In the pig one can also say that solitary cells become detached from the inner cell-mass and appear to be forerunners of the entoderm. Also, in abnormal blastocysts where there is a good trophoblastic wall, but no inner cell-mass, the absence of entoderm cells would indicate the necessity of an inner cell-mass. That it is difficult in the pig to find sections demonstrating the detachment of entoderm cells from the inner cell-mass is probably to be explained by the brief duration of the phenomenon and by the small number of cells that are so shed. The small number of cells composing the inner cell-mass precludes any large contribution for such a purpose, and it is quite possible that two or three cells would be sufficient. A count was made of the cells in specimen No. 283–2, one of the most mature eggs in the litter, and though such a count is subject to some error the proportionate representation must be fairly close. The result was as follows:

Cell Count, blastocyst 6 *days* 20 *hours*

Inner cell-mass	20–24 cells
Entoderm	80 "
Trophoblast	470 "

At the time of detachment, and more especially immediately following, there appears to be an active proliferation of the entodermal cells, which process is well shown in specimens No. 283–3 and No. 283–7 as described above. This is the time one finds spherical cells with marked nuclear activity. Such cell clusters are more commonly found near the inner cell-mass,

but scattered cells may lie at some distance away. When more mature entoderm cells make their appearance with long processes, these are found adhering to all parts of the blastocyst wall. Often one could not say but that they were arising directly from the trophoblast. However, in view of what has gone before one must not run the danger of being misled by such appearances without more satisfactory proof.

It is not until the entodermal lining is nearly complete that one can speak of a bilaminar blastocyst. The eggs we have just been considering, after fixation, have a diameter of 0.2 to 0.3 mm. By the time they acquire a diameter of 0.6 mm. the entoderm can be recognized as fairly complete and appears as a very thin membrane, and what interruptions exist in it seem to be due to fixation and shrinkage. This stage of the early bilaminar blastocyst is represented in figures 51–54, plate 4. The trophoblast consists of a thick wall whose single layer of cells is swollen with fluid and stands off in sharp contrast to the inner cell-mass. There were four eggs studied in this litter (No. 382), and they were all fairly distended with fluid. The accumulation of the segmentation-cavity fluid had caught up with the growth of the trophoblast which is doubtless to be credited to the functional activity of the trophoblast itself. The swollen character of the trophoblast in figure 52 is perhaps the histological picture of this functional activity.

The character of the entoderm in a slightly larger bilaminar blastocyst (0.8) is illustrated in figures 56, 55′, 58. The nuclei are more numerous and from the outset appear more thickly assembled directly under the inner cell-mass than elsewhere in the blastocyst. A surface view of the egg, near the dark appearing inner cell-mass, is shown in figure 55′, plate 4, and it will be noted that the wall is composed of larger and smaller cells. The latter are trophoblast cells and the larger are chiefly entoderm and in some cases trophoblast cells undergoing proliferation. An instructive picture is obtained in tangential sections of the wall, such as shown in figure 58, plate 4. Here one can readily distinguish the nuclei of four or five entoderm cells. The delicate membrane which more or less completely fills in the intervening space is of course lost. Contrasted to these is the overlying trophoblast whose closely fitted cells constitute an intact membrane.

GERM-DISK

That part of the egg that is destined to form the embryo is the last to differentiate. As the trophoblast begins to take definite form it can be distinguished from the slowly dividing primitive cells which, due to the fact that they tend to be elbowed away from the surface by the trophoblast, are known as the inner cell-mass. From this inner germinal mass additional trophoblast cells are given off, in varying numbers in different animals until a complete trophoblastic wall is provided for the egg. At about this time a few cells are separated off from this same inner cell-mass to become the ancestors of the entodermal system. What is left gives origin to the germ-disk. The essential steps in the differentiation of the germ-disk are shown on plate 4, figures 52, 54, 56 and 57. These sections are taken from eggs measuring as follows: figure 52, 0.6 mm.; figure 54, 0.6 mm.; figure 56, 0.8 mm.; and figure 57, 2–3 mm. A gross view of the first three of these eggs, while still intact, is shown at the left of the respective sections.

On studying the inner cell-mass of the 0.6 mm. egg one sees evidences of cell-proliferation, but the mass still retains its primitive appearance and is sharply marked off from the overlying trophoblast, the so-called Rauber's layer. In the 0.8 mm. egg one finds active nuclear division, some increase in the size of the inner cell-mass and a little thinning out of the overlying trophoblast. In the section of the 2–3 mm. egg one can now see that the formless inner cell-mass has differentiated into a single-layered plate of cylindrical cells actively proliferating. With this change the overlying trophoblast disappears, apparently by retraction to the edges, allowing the germ-disk to come to the surface with the trophoblast adherent to it around the periphery of the disk.

In this particular egg, figure 57, two trophoblast cells have become detached and are left behind near the center of the disk. Similar remnants of Rauber's layer were described by Weysse (1894) in his pioneer study of the pig egg. This sort of thing is not to be confused with the condition described by Keibel (1902) for the deer, where there occurs a definite ectodermal cavity. This specimen was chosen for illustration because it shows well these structures just at the time the germ-disk becomes exposed to the surface and when it acquires the dignified status of embryonic ectoderm.

In the fresh living egg the trophoblast is slightly opaque and the germ-disk is quite transparent, giving the appearance of a minute hole in the blastocyst wall. When fixed, however, the germ-disk becomes opaque and its appearance in a series of blastocysts of varying sizes is shown in figures 59–65, plate 5. These photographs are all made at the same enlargement. During this period there is an increase in the size of the germ-disk, but still more so of the trophoblastic wall. The growth of the latter soon leads to an entire change in the form of the egg, as will be described under the next heading.

FORMATION OF ELONGATED CHORION

The chorions enlarge and remain spherical for a brief period, but they soon become slightly collapsed or wrinkled; note, for example, the small specimens in figure 41, plate 3, which are already considerably wrinkled. The relative amount of contained fluid, however, is apparently quite variable; older embryos may contain a sufficient quantity to distend them. Thus, spherical vesicles (figs. 51, 53, 55, plate 4) may be seen which have a diameter of 1.5 mm. They then enlarge rapidly and once more become wrinkled (fig. 65, plate 5). If the largest wrinkled vesicles were distended they would still be spherical and have a diameter of about 5 mm. After this they begin to elongate, and the process goes on at a very rapid rate producing a remarkably long thread-like tubular chorion. During this early elongation the chorion shrinks considerably in diameter, but the two ends soon enlarge giving a dumb-bell appearance to the vesicle. Thus a vesicle 12.4 mm. in length (fig. 71, plate 6) has a diameter of 2.5 mm. at the enlarged end, whereas the diameter of the middle part is 1.4 mm. The diameter of the chorion, except for the ballooned ends which progressively expand, is further reduced until the tube is more than 100 mm. in length. The growth of the embryo then produces an enlargement in the middle of the chorion which otherwise has a more or less uniform diameter until the club-shaped ends are approached. After the somites begin to be marked off the chorion in the region of the embryo is thinner than in the parts beyond, and care is required in removing the specimens in order to preserve them with the membranes intact and the embryo uninjured. The absolute length of the chorion is greater when the embryo is in the somite stages than it is later; Bonnet has recorded the length of the 17-day vesicle as 1.4 meters. After the embryo attains a length of 5 mm. or more, there is an actual reduction in length of the chorion, although the surface for absorption and secretion is tremendously increased due to the marked increase in width.

PATHOLOGICAL CHORIONS

Occasionally the washings from a uterus will show fragmented chorions. The last four figures on plate 11 are examples of such specimens. Sometimes the chorions are broken up into irregular masses, some often having smooth areas on the surface or a small portion may even be nearly spherical. A portion of a chorion may be found (fig. 117) which is greatly flattened and marked with more or less circumscribed elevations and depressions. It is possible that these bizarre patterns are formed by the agglutination of fragments of a defective chorion, but that they may be produced by the indentation and even splitting of the chorion is indicated by specimens in which elongated perforations are present, and besides irregular villous processes have been found on a few defective chorions which is taken as further evidence that the bizarre arrangements may be formed *in situ*.

The embryonic area is usually located at about the middle point of the chorion, although in several specimens examined the disk was found considerably removed from this position. Thus the four embryos shown in plate 6, figure 68, are not symmetrical as regards the position of the embryonic area. The disk in the largest chorion is placed below the middle by a space which is 5 per cent the total length of the chorion. The other members of the litter have their embryonic spots located 13, 16 and 17 per cent respectively from the middle point. From one uterus nine embryos were recovered, ranging in length from 27 to 383 mm., a variation much greater than is usually found, and the displacement of the embryonic area is indicated by the values, 0, 1, 7, 10, 13, 25, 29, 39 and 44 per cent. Some defective embryos are to be expected in every litter, 20 to 25 per cent after segmentation stages according to Corner (1923), but since the variation in size amongst the members of the lot just mentioned is so great the question arises whether the specimens are normal. The first five embryos appear to be normal, whereas the remaining ones are

slightly atypical. The specimen showing a displacement of the embryonic area of 25 per cent has a thin asymmetrical disk. The next one has a few villous-like outgrowths which mark it as an abnormal embryo. The one showing 39 per cent displacement has an embryonic area which is quite thin and invaginated. The last embryo (44 per cent) shows a defective embryonic area very near one tip of the chorion. Thus the four chorions showing the greatest displacement of the embryonic area are slightly defective. If the area is too far removed from the middle point, it can apparently be regarded as abnormal. Such embryos would probably not proceed very far in their development, and one should not expect therefore to find a well-formed embryo attached near one end of a chorion.

DEVELOPMENT OF THE EXTERNAL FORM OF THE EMBRYO

PRIMITIVE GROOVE

In a previous section a description is given of the origin of the germ-disk, how it becomes a superficial plate fitted in and continuous with the trophoblastic wall of the vesicle. With the elongation of the chorion we have seen that the germ-disk retains an approximately central position with respect to the two ends. The figures 72–78 on plate 7 show this middle region in a series of specimens with the germ-disk, and all are reproduced at the same enlargement, which is the same and should be compared with figures 70 and 71, plate 6, and figures 59–65, plate 5. It will be seen that with the elongation of the chorion there has been a great reduction in its diameter. On the other hand, there is a very definite increase in the diameter of the germ-disk. This growth is already noticeable in the youngest of the series (fig. 72). In both figures 72 and 73 the disk is round and in the later stages becomes oval and then more and more elongated.

At this point reference must be made to the detailed description of the development of the germ-disk published by one of the authors in the preceding volume of the Contributions to Embryology (Streeter, 1927). That paper describes the histogenesis of the germ-disk, the formation of the primitive streak and the origin from it of the mesoblast. It also describes Hensen's node as it is found in the pig, and how giving origin to the notochord appears to be the particular purpose of the node. These matters will therefore be omitted here, and the reader is referred to that paper, which in that respect constitutes a supplement to the present treatise.

The elongation of the ectodermal disk seen in figure 74 is due to the active proliferation of its cells in the region of the primitive streak. At this time such an area can be recognized, constituting a caudal pole of germinal cells from which the mesoblast takes origin. A thin mesoblastic membrane can be seen through the wall of the chorion. One soon sees a sagittal crease in the caudal half of the disk, the primitive groove, which is illustrated in figures 75–77. These photographs were chosen because they show its haphazard position as regards the axis of the chorion. It should be noted that the primitive groove appears later than the primitive streak, and it will be seen that it subsequently disappears though the primitive streak continues in its functions as the germinal end of the embryonic disk. The primitive groove in reality is but a temporary phenomenon in the pig, and the mechanics of its production is perfectly evident, that is, a localized acceleration in the proliferation of the germ-disk cells that border it.

NEURAL GROOVE

At about the stage pictured in figure 77 it is possible to identify a new center of germ-disk activity in the center of the disk and just anterior to the primitive streak, the so-called Hensen's node. Coincident with its appearance and the laying-down of the anterior end of the notochord a new groove is formed in the anterior half of the germ-disk marking the anterior end of the definitive body of the embryo. This is the neural groove whose early appearance is shown in figure 79. The fact that it corresponds in time and position so exactly with the notochord is of significance. If Hensen's node be regarded as the seat at which the notochord is being separated and proliferated from the germ-disk, then the neural groove represents the path along which this has happened. In that sense it may be said to be that part of the germ-disk from which the notochord cells have been taken away.

The neural groove soon becomes more pronounced, due to the growth of the disk on each side of it. Figures 80 and 81 show the embryonic region as it is commonly seen at this

time. It varies somewhat according to how flat the specimen is spread out. It will be noticed that the neural groove extends through the anterior half of the disk and caudal to it lies the thick rounded germinal end of the disk, creased more or less distinctly in its center by a primitive groove and underneath pouring out the proliferating cells of the primitive streak. This same appearance persists at the caudal end of the embryo throughout the later stages, though it becomes relatively smaller. It represents the primitive streak proliferation and somewhat like a root tip constitutes the growing end of the embryo.

Figures 82 and 83 show that interesting period when the first somites form. The specimens are partially transparent and one can see opaque areas due to more dense mesoblast, particularly figure 83. Conspicuously outlined is the dense primitive streak region constituting the caudal third of the embryo. In front of this somite ridges extend forward on each side showing the indentations marking off the first two or three somite pairs. At the anterior end are the outlines of the flaring halves of the primitive brain-plate. It will be noted that the latter is elevated from a more ventral lying cardiac shelf.

Almost as soon as the outlines of the brain-plate can be recognized, one can begin to recognize individual brain structures, and it is evident that they are definitive from the outset. Particularly prominent, as shown in the human by Bartelmez (1922 and 1926), are the bilateral folds of the neural groove resulting from the active proliferation of the cells of the acoustic complex. The flaring-out of the neural walls in this region are plainly seen in figure 84. This sense organ, probably representing its vestibular part, is the first one to claim our attention and so may be regarded as the most primitive. It is soon followed by the optic, as we shall presently see. The specimen is curved transversely around the chorion so that the primitive streak region is obscured from view. When the caudal end is brought into view it is like that in figure 85.

The further history of the neural groove and the closure of its edges to form a neural tube can be traced on plates 8 and 9. It is especially instructive to trace the acoustic complex which is so readily identified by the bending-out of the neural walls. It shows well in figures 87, 89 and 92, and can be still seen in figures 98 and 99, plate 9. In the latter two a similar lateral flare of the walls with a central pocket occurs oral to the acoustic level, and this marks the region of the trigeminal nerve. These constitute important landmarks, and from this time on satisfactory orientation of the various brain levels is made possible.

FORMATION OF AMNION

The amnion in the pig is formed from folds in the extra-embryonic portion of the somatopleure. These folds rise up and surround the embryo similar, in a general way, to the process in the sauropsida. The embryonic area remains entirely exposed until after the primitive streak and early neural folds are developed. The beginning of an upward bending of the somatopleure at the periphery of the embryonic plate occurs just before the first somites are established. There is an early but distinct amniotic elevation surrounding the embryo shown in figure 79, plate 7. The mesoderm in this embryo has been reconstructed by Streeter (1927) and is illustrated in his figure 56, plate 6. The length of the primitive streak is yet approximately two-fifths that of the total length of the embryonic area. No somites have become delineated, but they soon will be since the embryo shown in figure 83, plate 7, has three somites, although the amnion is no further developed than in the embryo just mentioned. The later development of the amnion is shown in plates 7, 8 and 9. It is seen that the head and tail folds do not show themselves in advance of the lateral folds, but they all start up at about the same time and rapidly approach each other and fuse over the dorsal region of the embryo. In fact, as pointed out by Keibel (1897), since the amnion grows up around the entire embryo it is not to be separated into the various named folds. In mammals the head fold of the amnion is described as not generally more prominent than the tail fold. Thus, in talpa, the tail fold is the first to become established (Strahl, 1906), and a similar arrangement is found in the dog: however, in the cat the head fold is said to be the first to appear (Bonnet, 1901).

The amniotic umbilicus, or region where the amniotic folds have not yet fused, is of large caliber in embryos of about the 8-somite stage, figure 91, plate 8, extending from about the third to the eighth somite. The umbilicus is rapidly reduced in caliber, being converted into a slender tube whose length is already greater than its width in embryos possessing about ten

somites. It grows in length and at the same time its attachment appears to move cephalad so that in 5-mm. pigs the remnant of the structure is seen as a filament stretching out, in most cases, from that portion of the amnion which covers the dorsal region of the head. The amniotic attachment of the strand is not at all constant; it may be located opposite the side of the head or neck, or, as is more often the case, in the mid-dorsal plane. The cephalic position, however, is characteristic for mammals (Strahl, 1906).

SOMITES, EAR, EYE

The somites begin to appear at about the stages shown in the litter mates, figures 82 and 83, plate 7. These embryos were studied by Streeter (1927), and reconstructions of the mesoderm are shown in his figures 58 and 59. The age difference between these embryos is only slight, as is indicated by the condition of the primitive streak, the notochord and the pericardial cavity. However, there are no somites marked off in the younger embryo, whereas the older one shows two completed somites and the third is partially separated from the somite ridge. The succeeding somites are laid down as the embryo elongates caudally, as shown in plates 8, 9 and 10.

In embryos with about 8 to 9 somites the neural folds approach and lie in contact with each other from the acustico-facial region to a point a short distance beyond the somite ridge. An actual fusion of the folds very soon occurs in the region just below the acustico-facial enlargement in the brain.

Most of the somites produce elevations on the dorsal surface of the embryo, making it possible to count them while the embryo is yet intact. The first somite soon becomes more deeply buried in the surrounding mesenchyme so that it is not easily located in reflected light: likewise the last caudal somites may not be so evident in surface views. However, when the beam of light is favorably directed on the various regions of the embryo all the somites can be located. In the younger specimens the somites may be studied to advantage with transmitted light, using opal glass as a diffusing screen. When examining the embryos by this method the somites are rendered more apparent by the addition of a little glycerine to 80 per cent alcohol. Rarely can all the somites in an embryo be identified in a photograph on account of the curvature of the embryo which does not permit a favorable illumination of every somite at any one time. However, under the binocular microscope the embryo can be posed to bring out the somites in one region after another.

That portion of the neural plate from which the acoustic complex is derived exhibits active proliferation of cells with resultant bending of the neural folds at a time when the first somites are being demarcated and before any evidence can be seen of the optic apparatus. The otic plate, derived from the skin ectoderm, however, comes somewhat later. A small area of the ectoderm in the hind-brain region on the dorso-caudal portion of the hyoid swellings increases slightly in thickness after the 11-somite stage, producing the first indication of the development of the otic vesicle. The beginning of the invagination of the plate occurs in embryos with 16 to 19 somites. In the 20-somite specimen, figure 108, plate 10, there is a shallow otic pit, the edges of which have not yet started to close in. The caudo-median portion of the pit is the deepest and the walls here are beginning to fold over in the 23-somite embryo, figure 109. The otic vesicle is then gradually completed by the growing together of the walls around the whole periphery of the pit. The diameter of the opening is about half that of the entire vesicle in the 26-somite pig, and the closure is completed at about the 28-somite stage.

The optic primordium appears a little later than the otic primordium, the latter referring to the alterations in the medullary walls giving origin to the acoustic segment. The invagination of the optic primordium has not yet been initiated in the 8-somite embryo (fig. 100, plate 9). A depression is about to form, however, since in 9-somite specimens one usually finds deep optic sulci located near the lateral edges of the cephalic ends of the flat neural plates. Since the neural plates bend down at right angles at the mid-brain region, the sulci can be seen only when the embryo is studied end on. As the sulci become further invaginated a primitive optic vesicle takes form and can be seen shimmering through the ectoderm above them, much in advance of the closure of the neural folds. In 15- and 16-somite embryos (figs. 110 and 111, plate 11), the anterior neuropore is just forming and the optic vesicle has enlarged to a distinct dome-shaped structure, the vesicle being slightly oval in outline. The optic vesicles are large lateral swellings on the fore-brain

which give the greatest width to this part of the head. This and the subsequent stages of the vesicle, as it appears in cleared specimens, is illustrated in the paper by Heuser, 1923, plates 1 and 2. It is not until we come to embryos of 6 mm. that the vesicle is finally exceeded in size by expanding cerebral hemispheres.

ALLANTOIS

The caudal end of embryos with four to five somites (fig. 88, plate 8), is slightly flared out, this being the first indication of the mesoderm of the allantois. The allantois entoderm grows into it and rapidly expands. The evagination from the hind-gut is first definitely seen in embryos with about six somites. Often the border of the enlarged caudal end is slightly frilled in the early stages, as seen in figures 92 and 93. The allantois of the small embryos can be studied to advantage in unstained specimens which have been cleared in wintergreen oil. With a piece of black paper beneath the dish and a beam of light striking the embryos horizontally the external surface of the allantois, as well as other structures, stand out in clear relief under the binocular microscope; and when light is directed up through the embryo by means of a white cardboard reflector the extent of the entodermal cavity is clearly seen.

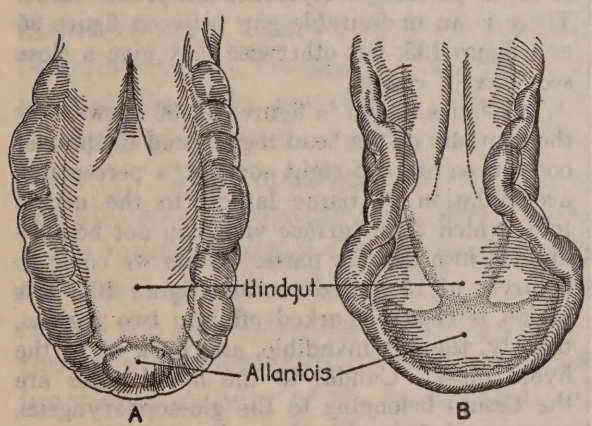


FIG. 8—Development of the allantois, as seen in the transparent embryo. A, 7-somite specimen (No. 215–2) same as shown in figure 97; B, 10-somite specimen (No. 442). Enlarged, 42 diameters.

In text-figure 8 is shown the outlines of the allantois drawn from embryos in wintergreen oil. The embryo in text-figure 8, A, has seven somites and is the same specimen pictured in figures 95 and 97. The entodermal part of the allantois is only a small evagination from the hind-gut. Text-figure 8, B, is a drawing of the caudal end of a 10-somite embryo. The lumen of the allantois has expanded into a barrel-shaped cavity, the tip ends of which are slightly constricted indicating the horns which are characteristic of older embryos. Compare with older stages shown in figures 108 and 114. In acquiring the older characteristics the cephalic border of the allantois gradually grows free from the embryo, thereby producing the horns. These are visible for the first time at about the 20-somite stage, figure 104. The cavity of the allantois continues to enlarge rapidly and forms a crescentic sac which hangs down ventrally from the caudal end of the embryo, as seen in the photographs on plate 10.

A study of the later development of the allantois and its relation to the uterus was made by Heuser, 1927. It was shown by him that the allantois becomes very vascular and as it expands it applies itself to the inner surface of the surrounding chorion. At about the 7-mm. stage (see Heuser, figs. 1 and 2) the actual fusion of these two membranes begins; during the next three weeks the growth of them is tremendous and the fusion between them spreads to the tips. The growing embryo is thus provided with an adequate respiratory and nutritive mechanism by this enlargement of the allanto-chorion which does not penetrate but lies in close apposition to the uterine epithelium.

CLOSURE OF ANTERIOR NEUROPORE

The neural folds at the 8-somite stage, figure 100, plate 9, appear to be fused together in a short zone only between the trigeminal and otic segments of the brain. In front of this point of union the folds slant apart, but caudal to it they lie in more or less intimate contact as far as the caudal end of the somite ridge. The specimen was not sectioned, but a careful study under the binocular revealed no evidence of actual fusion throughout this stretch, although it was imminent. In figure 101 several places appear to be fusing at the same time. In figure 102 the whole region has blended and we can speak of a neural tube. The specimens shown in figures 100–102 differ but little in development, and it is evident from them that the process of fusion of the medullary folds is an abrupt one and occurs over a wide area of the tube simultaneously. Subsequent to this first extensive blending the process continues orally and caudally more gradually, leaving

the two ends still flaring open as the anterior and posterior neuropores.

The closure of the anterior neuropores can be followed in figures 110–113, plate 11. It is at the lateral edges of the front end of the neural plates that the optic sulci appear at about the 9-somite stage. The front end of the head is nearly flat and is entirely covered by the spread-out neural plates. The optic pits at their lateral edges drop down abruptly and elsewhere slope gradually to the surface of the neural plate. They are directed caudally, that is, downward when the embryo is viewed head on, with a slight lateral and dorsal tilt. As the folds grow they begin to roll over everywhere more rapidly than at the extreme median point, while at the same time the fusion in the dorsal midline is slowly creeping upward. Looking at the oral surface of the head in a series of embryos, figures 110–113, it is seen that the deepening optic pit on each side soon becomes hidden below the ledge of neural fold. As the optic sulcus becomes converted into the pit it soon attains a depth which is greater than its open mouth. The long axis of the pit, which at first is vertical to the flat end of the head, shifts with the growth and rolling-in of the neural folds so that it gradually becomes inclined toward the mid-plane. The pit also grow slightly caudad while the neural folds are completing their closing-in process.

If a large number of embryos at the critical stages were studied it would probably be found that there is a fairly close correlation between the time of closure of the neural folds and the development of the various other structures of the embryo. Specimens of the stage we are considering show, however, that a considerable variation may exist. It is true that a rather consistent picture is given of the closure of the folds, but embryos that are of about the same stage of development when judged by the number of somites, the extent of fusion of the neural folds, and the development of the allantois, may show considerable differences in the extent of closure of the anterior neuropore and give quite unlike pictures of the front end of the head. It seems that the amount and kind of tissues may be the same in two embryos, but the actual bending-over of the folds be different.

The final closure of the anterior neuropore occurs in embryos of about the 20-somite stage. The specimen shown in figure 113 is just in the last steps of closure. Sections through such a specimen show a sharp line of demarcation between the thin skin ectoderm and the thicker neural wall, and it is evident that the curving-in of the latter is the effective force in the closure. The photograph of the specimen shown in figure 112 gives a misleading impression of the neuropore. In reality it is a definite slit-like opening larger than that in figure 113.

It may be added that the posterior neuropore closes very soon after the anterior. In the 23-somite embryo, which stage is illustrated in figure 108, it is already closed. In an embryo of 20 somites, figure 107, it is still open.

BRANCHIAL ARCHES

In all vertebrates a prominent part in the development of the face is taken by a series of prominences and ridges seen on the ventrolateral surface of the head. The most oral of these is known as the mandibular arch, the maxillary process lying just in front and above it. Caudal to it is the hyoid arch with two or more similar arches in turn forming caudal to it. Together with the hyoid the latter are usually spoken of a branchial or visceral or gill arches. The part that these take in influencing the surface anatomy of the embryo is shown in a series of photographs on plates 8 and 10, figures 94–96 and 105–108. These were taken as far as possible to represent left profile views. There is an undesirable gap between figure 96 and figure 105, but otherwise they give a close sequence of events.

The series shown in figures 94–96 shows only the contours of the head region, and in the last one, an embryo of eight somites, a perceptible accumulation of tissue lateral to the neural folds which from surface view can not be subdivided into definite parts. When we come to embryos of 15 paired somites, figure 105, this region is clearly marked off into two regions, namely, the left mandible, and back of it, the hyoid mass. Caudal to the hyoid mass are the tissues belonging to the glossopharyngeus, vagus and hypoglossal territory. The next embryo, figure 106, is much like it. The mandible is more definitely marked off, and one can begin to see an indication of the maxillary process. Above these is an elevation caused by the trigeminal nerve. The hyoid mass is marked in its upper part by the thickening of the otic plate.

In coming to embryos of 20 somites we find a definite maxillary process and a clearly out-

lined mandible. Blending with both of these is the trigeminal crest material from above. The hyoid mass is now separated into a dorsal part belonging to the acoustic complex and a ventral part. It is only this latter part that can be spoken of as the first branchial arch. In other words, this branchial arch is a derivative of a still more primitive structure.

The transition to the typical branchial-arch picture is provided in figures 108 (23 somites) and 109. It will be noted that the mandible retains its identity and on close examination its own characteristics. This is true also of the hyoid arch, although the latter bears closer resemblance to the arch forming back of it, which is doubtless to be explained by their mutual relations to the large vascular trunks coursing through them and to the presence of the intervening branchial membranes. One can see the origin of the post-hyoidal arch from the tissue-mass belonging to the occipital nerve group. The otic cup can be plainly seen in figure 108 and the closed-off otic vesicle can be seen through the transparent surface ectoderm, just above and caudal to the hyoid arch, marked off below by the shadow of the primary head vein. Certainly there is no true gill representative in any of these early stages in the pig, although there is abundant material belonging to the visceral apparatus.

SUMMARY

Based on abundant material the successive steps in the development of the pig egg have been traced from the one-cell stage to the time of the appearance of the limb-buds, and the principal morphological changes that terminate in a definitely organized embryo are herewith presented in the form of photographs. Except for that of the first week the age of the material was not known, being acquired from the uteri of pigs of unknown origin. The copulation age was known for the eggs of the first week, these having been obtained from sows that had been bred for this particular purpose.

On following the cleavage of the egg it was found that structural differentiation of the trophoblast cells begins as early as the fifth day after insemination, in eggs of about 16 cells. They lose their primitive form of large round cells with distinct boundaries and become flattened out in a continuous single-layered membrane against the zona pellucida. Coincident with this the fluid of the segmentation cavity begins to accumulate, which is assumed to be the result of their functional activity. The trophoblast cells are the most precocious of the cells that compose the egg and soon constitute a membrane completely enclosing it. The inner cell-mass is the residual part of the egg and for a time retains its primitive character. After the last trophoblast cells have separated from it, there is left those cells that give rise to the germ-disk and its underlying entoderm. As a corollary, it may be said that the segmentation cavity is a product of the precocious differentiation and functional activity of the trophoblast; it is a purely trophoblastic structure and a necessary mechanism of implantation, and an essential step in the development of the membranes that are to enclose the embryo.

The storage of fluid, as an intercellular pool, within the space walled off by trophoblast, converts the egg into a blastocyst and marks the beginning of its increase in size. At first the growth of the trophoblast wall outstrips the accumulation of fluid, and one then finds the eggs collapsed and wrinkled. As the fluid proportionately increases the eggs become spherical again. Finally the trophoblastic wall reshapes itself from a spherical vesicle to a long tubular thread. Though

the transitional stages are available for study the mechanism by which this is accomplished is not apparent.

The germ-disk is the last part of the egg to begin active differentiation. After arriving at the blastocyst stage the primitive inner cell-mass gives off one or more entoderm mother cells and at the same time commences active proliferation, becoming a definite ectodermal plate—the germ-disk or embryonic shield. From a limited number of entoderm cells, through proliferation, there is soon provided a continuous entoderm lining producing a bilaminar blastocyst. The entoderm directly underlying the germ-disk differs from that adhering to the trophoblast in its more active proliferation, the nuclei being larger and closer together.

The germ-disk is ordinarily found in the central part of the chorion. When it is removed from the center for a distance equivalent to more than 25 per cent of the total length of the chorion, the embryos are found to be defective. With the formation of the primitive streak the germ-disk becomes elongated and is found to lie in a haphazard position as regards the long axis of the chorion. The site of the proliferation that represents the primitive streak is therefore not determined by the axis of the chorion.

The formation of the primitive streak is the first evidence of polarity of the disk. From it arises the mesoblast which soon is assorted into various tissues of the embryo. The primitive streak takes the form of a thick rounded blunt end of the disk, creased more or less distinctly in its center by a primitive groove, and pours out ventrally its proliferating mesoblast cells. This same appearance persists at the caudal end of the embryo throughout the later stages, though it becomes relatively smaller. Like a root tip it constitutes the germinal end of the embryo.

The neural groove makes its appearance coincident with Hensen's node and the front end of the notochord, and corresponds in position exactly with the latter. If Hensen's node is accepted as the seat at which the notochord is being proliferated and separated from the germ-disk, then the neural groove represents the path along which this has happened.

The early differentiation of the acoustic apparatus follows the appearance of somites, and a little later the first evidences of the optic groove can be seen. In the body of the paper a brief description is given of these structures, as well as of the morphogenesis of the principal parts of the embryonic body.

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BIBLIOGRAPHY

ASSHETON, R. 1898. The segmentation of the ovum of the sheep, with observations on the hypothesis of a hypoblastic origin for the trophoblast. Quart. Jour. Micros. Sci., vol. 41, pp. 205–261.

———. 1898. The development of the pig during the first ten days. Quart. Jour. Micros. Sci., vol. 41, pp. 329–359.

BARTELMEZ, G. W. 1922. On the origin of the otic and optic primordia in man. Jour. Comp. Neurol., vol. 34, pp. 202–232.

———, and H. M. EVANS. 1926. Development of the human embryo during the period of somite formation, including embryos with 2 to 16 pairs of somites. Contributions to Embryology, vol. 17, Carnegie Inst. of Wash. Pub. No. 362, pp. 1–67.

BONNET, R. 1901. Beiträge zur Embryologie des Hundes. Anat. Hefte, vol. 16, pp. 232–413.

CORNER, G. W. 1921. Abnormalities of the mammalian embryo occurring before implantation. Contributions to Embryology, vol. 13, Carnegie Inst. of Wash. Pub. No. 276, pp. 61–66.

———. 1923. The problem of embryonic pathology in mammals, with observations upon intrauterine mortality in the pig. Amer. Jour. Anat., vol. 32, pp. 523–545.

HARTMAN, C. G. 1916. Studies in the development of the opossum *Didelphys virginiana* L. Jour. Morph., vol. 27, pp. 1–83.

———. 1919. Studies in the development of the opossum *Didelphys virginiana* L. Jour. Morph., vol. 32, pp. 1–142.

HEUSER, C. H. 1923. The branchial vessels and their derivatives in the pig. Contributions to Embryology, vol. 15, Carnegie Inst. of Wash. Pub. No. 332, pp. 123–139.

———. 1927. A study of the implantation of the ovum of the pig from the stage of the bilaminar blastocyst to the completion of the fetal membranes. Contributions to Embryology, vol. 19, Carnegie Inst. of Wash. Pub. No. 380, pp. 229–243.

HILL, J. P. 1910. The early development of the marsupialia with special reference to the native cat (*Dasyurus viverrinus*). Quart. Jour. Micros. Sci., vol. 56, pp. 1–134.

HILL, J. P. 1918. Some observations on the early development of *Didelphys aurita*. Quart. Jour. Micros. Sci., vol. 63, pp. 91–139.

———, and M. TRIBE. 1924. The early development of the cat. Quart. Jour. Micros. Sci., vol. 68, pp. 513–602.

HUBER, G. C. 1915. The development of the albino rat, *Mus norvegicus albinus*. Jour. Morph., vol. 26, pp. 1–142.

HUBRECHT, A. A. W. 1902. Furchung und Keimblattbildung bei *Tarsius spectrum*. Verhand. d. k. Akad. v. Wetensch. t. Amsterdam (Tweede Sect.), vol. 8, pp. 1–113.

KEIBEL, F. 1897. Normentafel zur Entwicklungsgeschichte des Schweines (*Sus scrofa domesticus*). Jena, G. Fischer.

———. 1902. Die Entwickelung des Rehes bis zur Anlage des Mesoblast. Arch. f. Anat. u. Physiol., Anat. Abth., pp. 292–314.

v. SPEE, F., GRAF. 1901. Die Implantation des Meerschweincheneies in die Uteruswand und seine weitere Entwicklung. Zeitschr. f. Morph. u. Anthrop., Bd. 3, pp. 130–182.

STRAHL, H. 1906. Die Embryonalhüllen der Säuger und die Placenta. Handbuch der vergleich. u. exper. Entwick. der Wirbeltiere, herausg. von O. Hertwig. Jena, G. Fischer.

STREETER, G. L. 1927. Development of the mesoblast and notochord in pig embryos. Contributions to Embryology, vol. 19, Carnegie Inst. of Wash. Pub. No. 380, pp. 73–92.

VAN BENEDEN, E. 1899. Recherches sur les premiers stades du développement du Murin (*Vespertilio murinus*). Anat. Anz., vol. 16, pp. 305–334.

VAN DER STRICHT, R. 1911. Vitellogenèse dans l'ovule de chatte. Arch. Biol., vol. 26.

WEYSSE, A. W. 1894. On the blastodermic vesicle of *Sus scrofa domesticus*. Proceed. Amer. Acad. of Arts and Sciences, vol. 30, pp. 283–321.

PLATE 1

Cleavage and formation of the segmentation cavity in the pig egg. The last four figures show early phases in differentiation of the trophoblastic portion of the egg.

Tubal eggs, one-cell stage, copulation age 2 days 3 hours.

Fig. 9, No. 388–3, ×180
Fig. 10, No. 388–4, ×180
Fig. 11, No. 388–B, ×470
Fig. 12, No. 388–A, ×470
Fig. 13, No. 388–3, ×180

Tubal eggs, two-cell stage, copulation age 2 days 3½ hours.

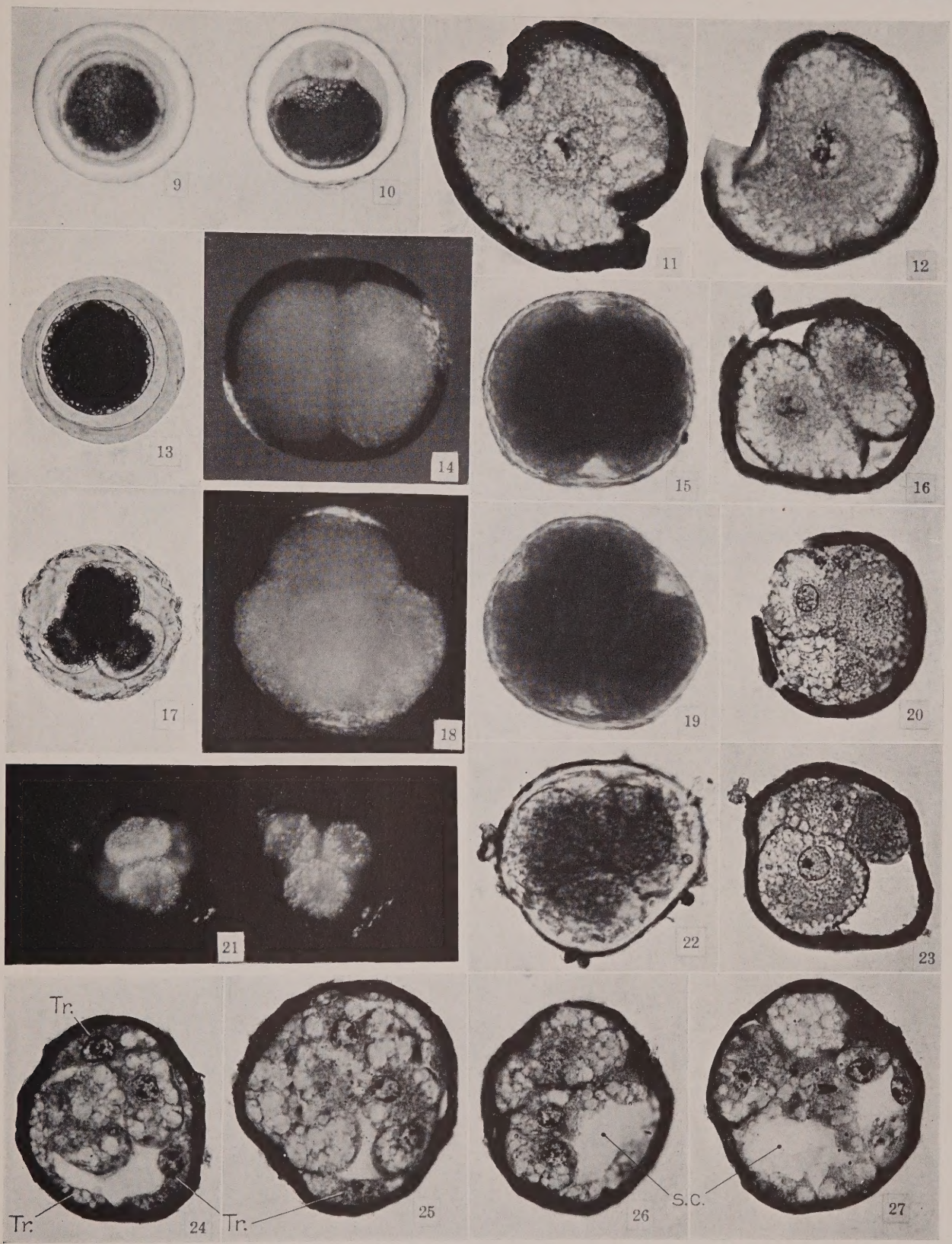
Fig. 15, No. 286–1, ×415
Fig. 14, No. 286–1, ×415
Fig. 16, No. 286–3, ×400

Tubal and uterine eggs, four- to eight-cell stages, copulation age 2 to 3¾ days.

Fig. 17, No. 389, ×180 (In salt solution)
Fig. 18, No. 285, ×400 (In 80% alcohol)
Fig. 19, No. 285, ×400 (In 80% alcohol)
Fig. 20, No. 285–3, ×400
Fig. 21, No. 389, ×180
Fig. 22, No. 281, ×400
Fig. 23, No. 281–3, ×400

Uterine eggs, early segementation cavity, 4 days 3½ hours.

Fig. 24, No. 284, ×500
Fig. 25, No. 284, ×500
Fig. 26, No. 284, ×500
Fig. 27, No. 284, ×500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Tr.
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S.C.
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PLATE 2

Small blastocysts showing separation of trophoblast from the inner cell-mass and in figures 32 and 39, entoderm mother cells.

Uterine eggs, copulation age 4 days 18 hours.

Fig. 28, Whole litter, No. 291, ×180
Fig. 29, No. 291–7, ×400
Fig. 30, No. 291–6, ×360
Fig. 31, No. 291–5, ×400
Fig. 32, No. 291–6, ×400

Litter of eight uterine eggs, copulation age 6 days 1¾ hours.

Fig. 33, Seven eggs of litter No. 287, ×180
Fig. 34, No. 287–6, ×400
Fig. 35, No. 287–8, ×400
Fig. 36, No. 287–3, ×400
Fig. 37, No. 287–3, ×400
Fig. 38, No. 287–X, ×400
Fig. 39, No. 287–2, ×400
Fig. 40, No. 287–7, ×400

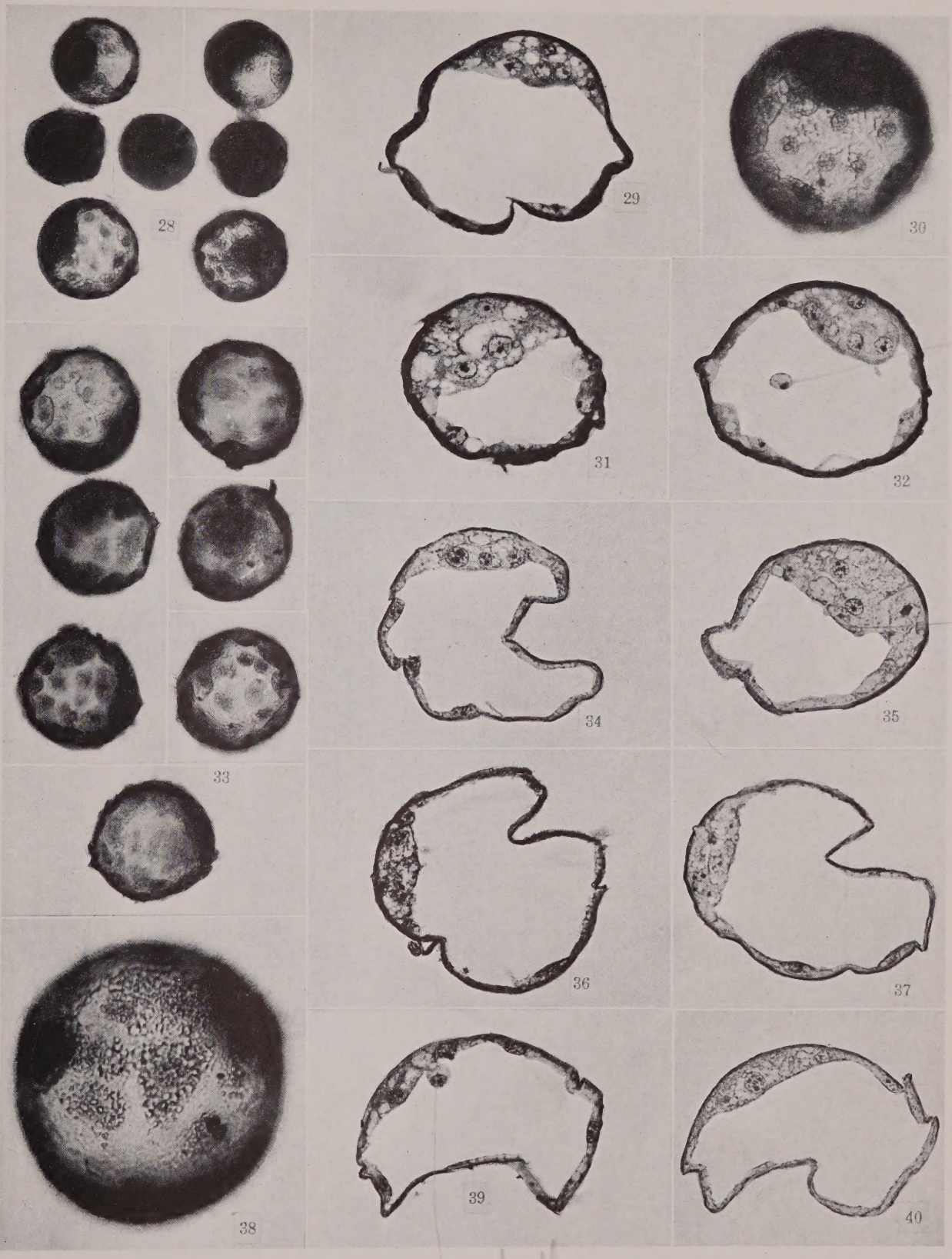
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PLATE 3

Enlarging blastocyst with numerous entoderm cells and definite germ-disk. All figures on the plate are from the same litter, No. 283, having a copulation age of 6 days 20 hours.

Fig. 41, whole litter, ×38
Fig. 42, surface view of egg, ×180
Figs. 43–50, typical sections, ×400

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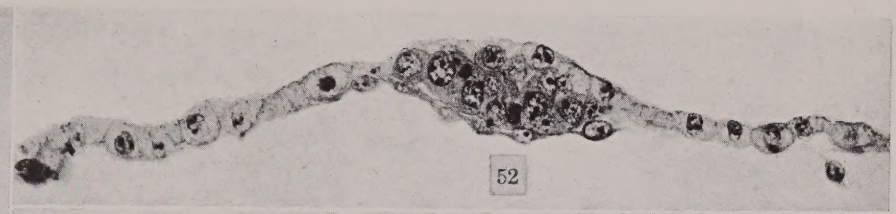
PLATE 4

The bilaminar blastocyst, showing stages in the differentiation of the germ-disk.

Fig. 51, No. 382–4, ×50
Fig. 52, No. 382–4, ×400
Fig. 53, No. 382–1, ×50
Fig. 54, No. 382–1, ×400
Fig. 55, No. 185–1, ×48
Fig. 55′, No. 185–1, ×150
Fig. 56, No. 185–1, ×400
Fig. 57, No. 206–1, ×400
Fig. 58, No. 185–1, tangential view of trophoblast and entoderm, ×200

51



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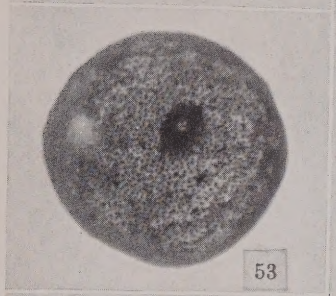

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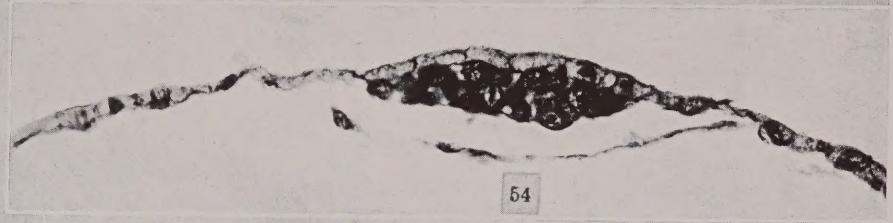

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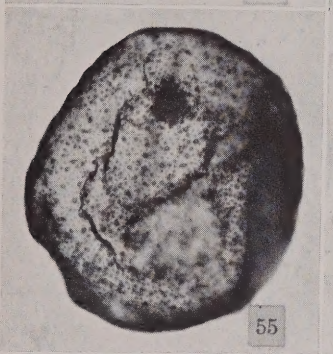

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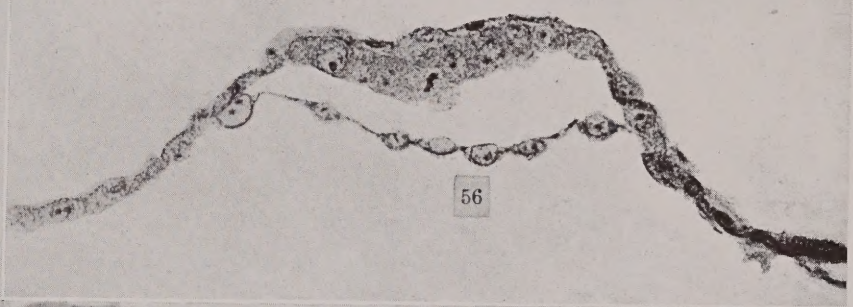

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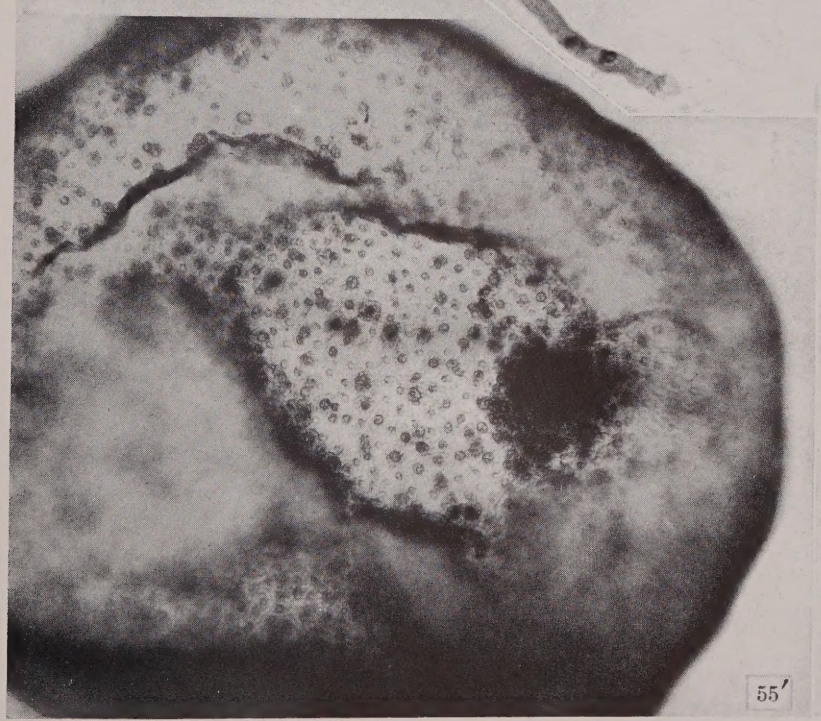

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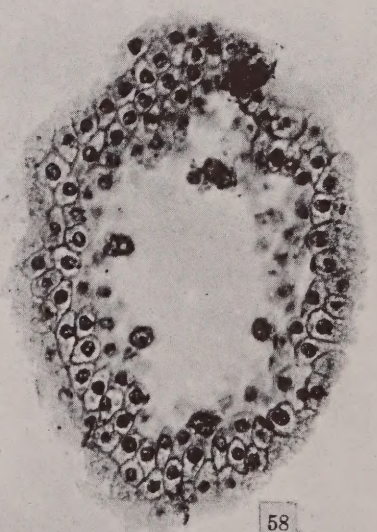

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PLATE 5

The enlarging blastocyst. These photographs are all made at the same enlargement (×12).

Fig. 59, No. 205–3
Fig. 60, No. 183–1
Fig. 61, No. 204–3
Fig. 62, No. 183–5
Fig. 63, No. 203–2
Fig. 64, No. 203–1
Fig. 65, No. 203–3

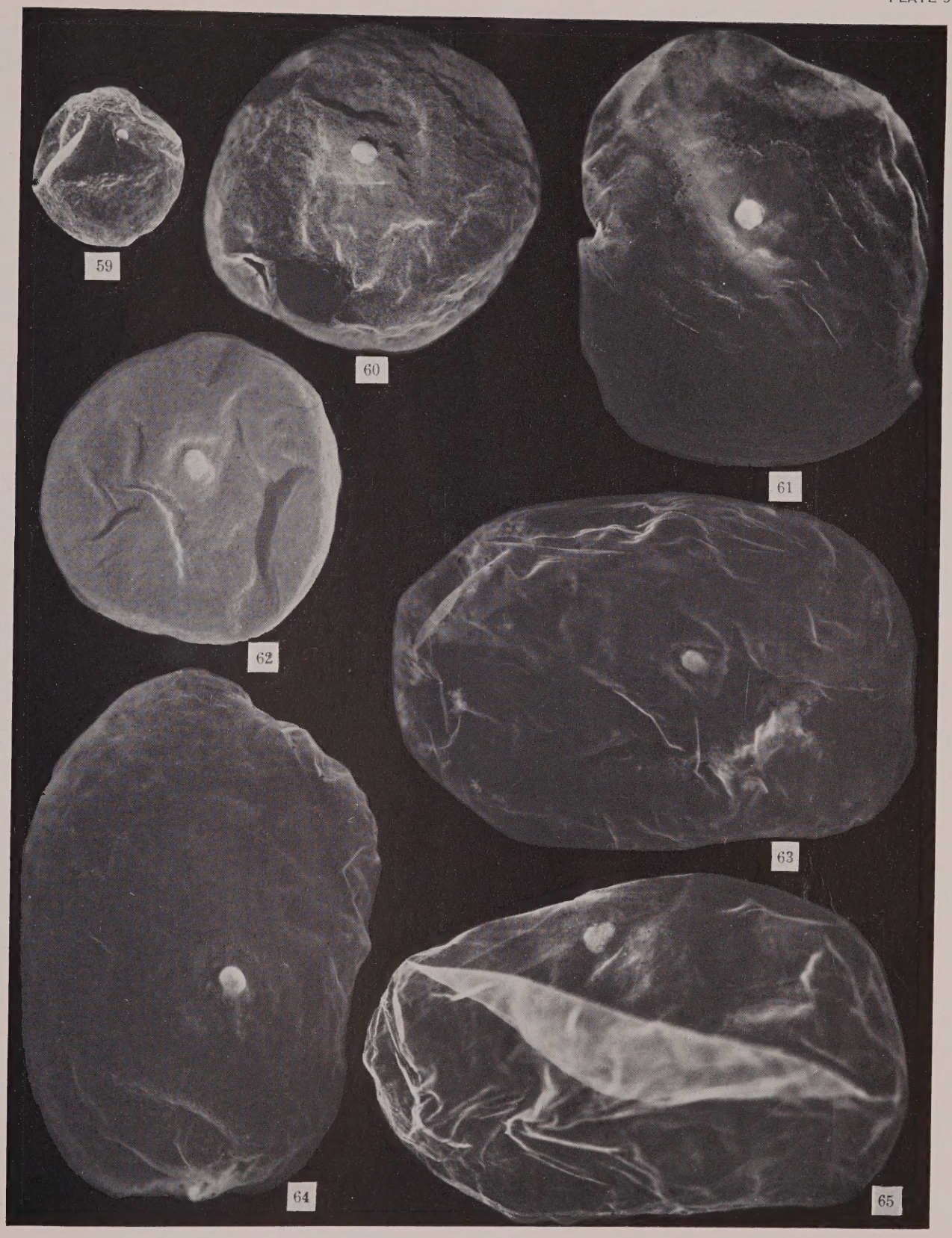
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PLATE 6

Three litters of eggs showing transitional stages from the spherical blastocyst to an elongated narrow tubular form.

Fig. 66, Litter (No. 264) of seven eggs showing beginning elongation. ×4.5

Fig. 67, Litter (No. 213) of seven eggs distinctly elongated. ×2

Fig. 68, Litter (No. 394) of four eggs slightly older. ×2

Figs. 69, 70 and 71, Detail of central part of three specimens shown in figure 67. Note the characteristic wrinkling of the trophoblastic membrance that occurs at the time of this rapid reshaping of the egg. ×12

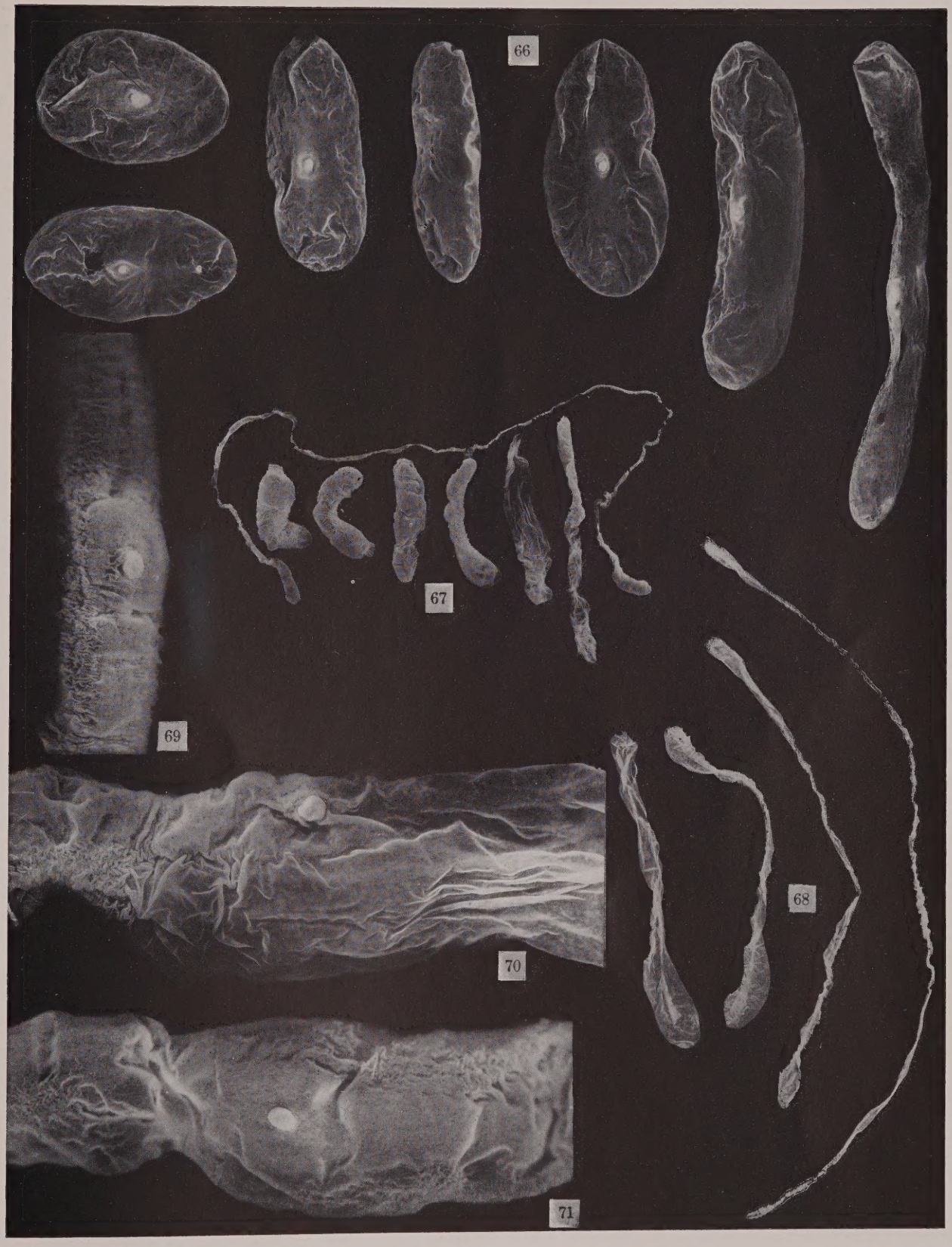
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PLATE 7

Differentiation of the Germ-disk

Specimens showing first appearance of the primitive groove and its haphazard position relative to long axis of chorion. Beyond the margins of the germ-disk the spreading mesoderm can be seen through the transparent wall. ×12.

Fig. 72, No. 160–33
Fig. 73, No. 179–2
Fig. 74, No. 179–1
Fig. 75, No. 169–4
Fig. 76, No. 169–5
Fig. 77, No. 181 (dorsal view)
Fig. 78, No. 181 (lateral view)

Specimens showing a neural groove. ×25

Fig. 79, No. 211–3
Fig. 80, No. 160–68
Fig. 81, No. 160–36
Fig. 82, No. 190–1

Early somite stages. ×25

Fig. 83, No. 190–2, 3 somites
Fig. 84, No. 214–1, 5 somites. Note acoustic region
Fig. 85, No. 190–3, amniotic fold

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PLATE 8

Differentiation of the neural folds and the beginning of their transformation into a neural tube. Such specimens show characteristic molding of the neural walls by which some of the more primitive parts of the brain can be recognized. In some of the specimens the amnion was removed, but it is well shown in figures 86, 90, 91 and 96. ×25.

Fig. 86, No. 166
Fig. 87, No. 214–1, 5 somites
Fig. 88, No. 164, 5 somites
Fig. 89, No. 195–1, 6 somites
Fig. 90, No. 168
Fig. 91, No. 158, 8 somites
Fig. 92, No. 158 (amnion removed)
Fig. 93, No. 176–2, 7 somites
Fig. 94, No. 257–1
Fig. 95, No. 215–2
Fig. 96, No. 268–1

86
87
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PLATE 9

Closure of neural tube and development of allantois. Figures 98–100 show clearly the parts of the neural wall belonging to the trigeminal and acoustic mechanisms. It will be noted that in late stages like figures 103 and 104 one can recognize the primitive streak region still functioning as a germinal area or growing tip, proliferating more tissues at the caudal end of the body. ×25.

Fig. 97, No. 215–2, 7 somites
Fig. 98, No. 163
Fig. 99, No. 162, 7–8 somites
Fig. 100, No. 268–1, 8 somites (S. 3, third occipital somite)
Fig. 101, No. 176–6
Fig. 102, No. 159
Fig. 103, No. 223–2, 18 somites
Fig. 104, No. 172–1

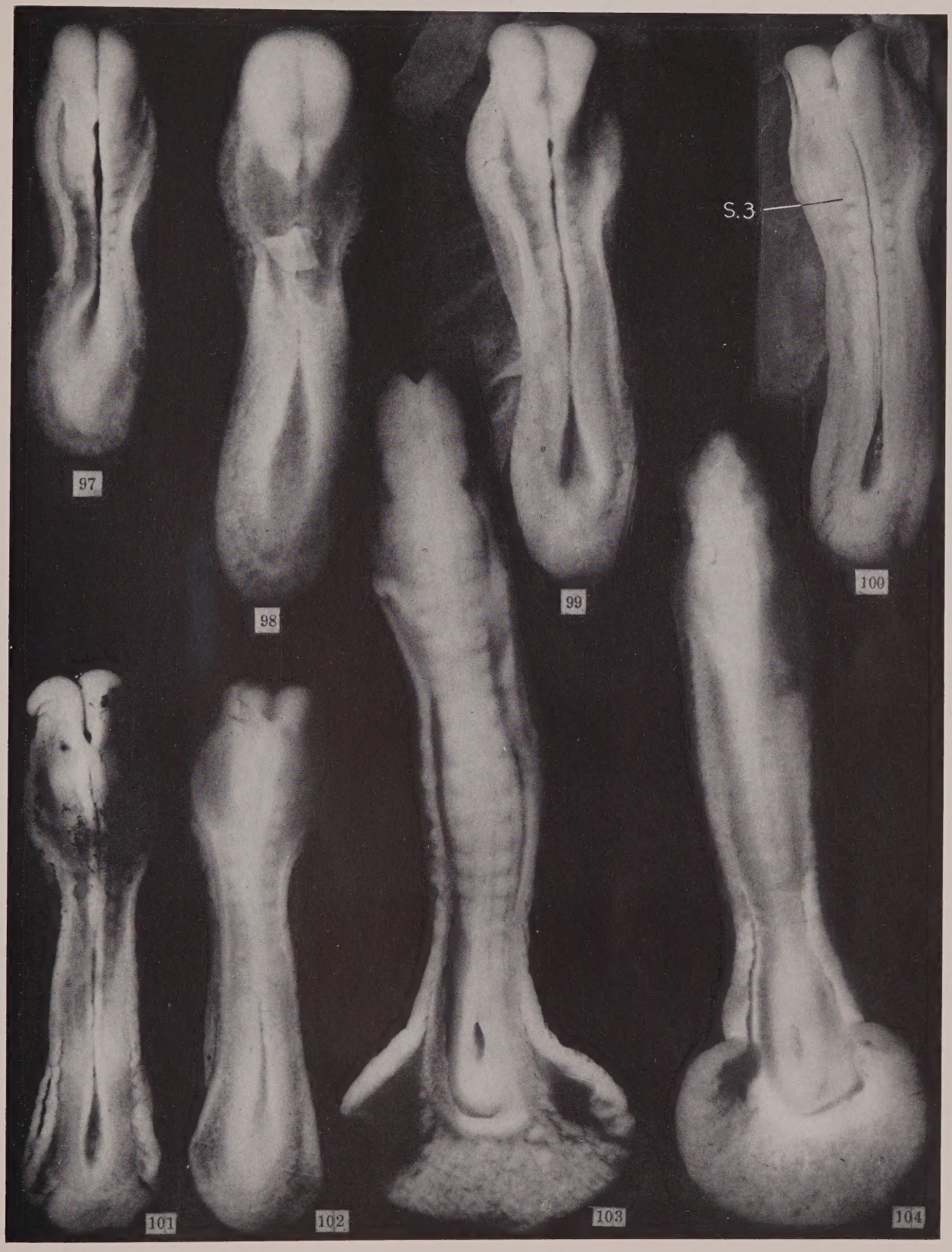
S.3
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PLATE 10

Development of the branchial arches. This series of profile views shows the development of the left mandible together with the maxillary process and their relation to the trigeminal nerve. Caudal to these is the hyoid prominence, the ventral part of which becomes the first of the branchial arches (facial). The dorsal part participates in the development of the acoustic complex. The depression in the otic plate can be plainly seen in figures 106 and 107. The tissues caudal to the hyoid elevation fall in the territory of the glossopharyngeus and vagus, and in figures 107 to 109 become segregated in the form of arches. The third occipital somite is marked S. 3. Enlargement ×25.

Fig. 105, No. 270–1, 15 somites
Fig. 106, No. 217–1
Fig. 107, No. 259–1, 20 somites
Fig. 108, No. 397–1, 23 somites
Fig. 109, No. 266–1

S.3
105
109
S.3
S.3
106
107
108

PLATE 11

Closure of the anterior neuropore. In figures 110 to 113 the yolk sac has been removed and the specimen posed so as to display an oral view of the head. The head in figure 112 is flexed further downward than the others. In reality the position of its cleft-like neuropore is the same as that in figure 113. In this latter figure a good view is obtained of the right and left mandibles, approaching each other in the middle line. The allantois will be noticed in these figures, and the one in figure 114 shows its structure just preceding its fusion with the chorion. Figures 115 to 118 are representative specimens of abnormal chorions.

Fig. 110, No. 223–1, 16 somites, ×25
Fig. 111, No. 270–1, 15 somites, ×25
Fig. 112, No. 217–1, ×25
Fig. 113, No. 259–1, 20 somites, ×25
Fig. 114, No. 160–79, ×4
Fig. 115, No. 219–1, ×5
Fig. 116, No. 396–4, ×6
Fig. 117, No. 396–2, ×6
Fig. 118, No. 396–1, ×6

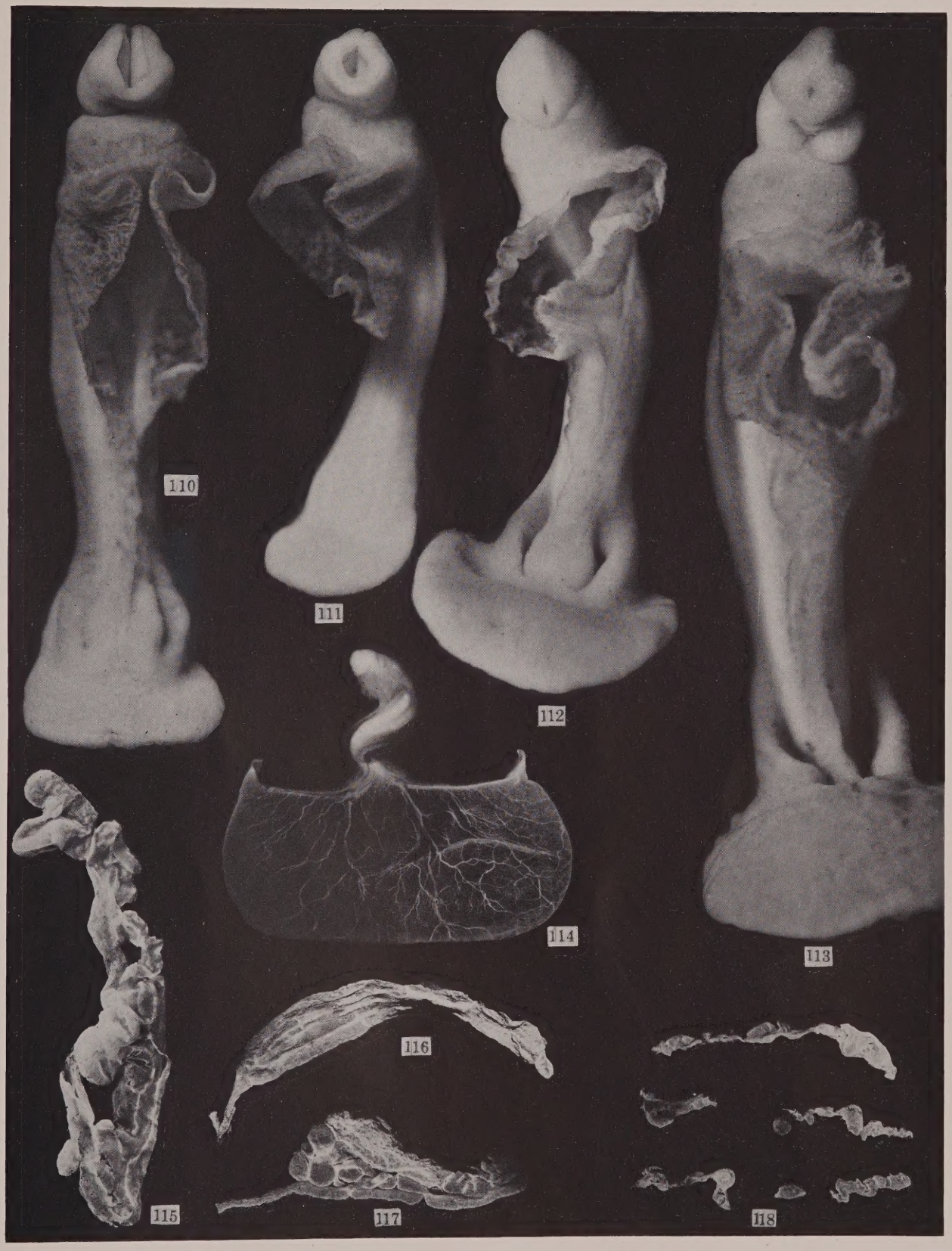
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PLATE 12 (Fig. 119)

This plate is designed to show the early differentiation of trophoblast (colored buff) in the mammalian egg. As compared to the rest of the egg it is precocious, and thus there occurs a fundamental separation of two kinds of material: *a*, cells that will form the implanting mechanism and fetal membranes, the so-called non-formative cells; and *b*, the more retarded and primitive inner cell-mass, the so-called formative cells. It is to be observed, however, that this latter group contributes cells in varying extent to the former, and it is the residual cells of this mass that are finally differentiated into the germ-disk and underlying entoderm.

These tracings are interpretations made from the publications of the following authors: Opossum, Hartman 1919; Rabbit, P. W. Gregory of the Bussey Institution; Guinea Pig, v. Spee 1901; Rat, Huber 1915; Pig, Assheton 1899; Sheep, Assheton 1899; Cat, Hill and Tribe 1924; Bat, van Beneden 1899; and Tarsius, Hubrecht 1902. The magnifications are brought to a common enlargement: viz., 150 diameters.

a
b
c
d
e
Opossum
Guinea Pig
Rabbit
Pig
Albino Rat
Cat
Bat
Sheep
Tarsius